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INFLUENCE OF ANDROGENS ON THE GROWTH AND METASTASIS OF THE BROWN-PEARCE EPITHELIOMA

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The chemical resemblance between "sex hormones" and carcinogenic substances of the phenanthrene series is ample justification for a thorough study of the effects of these "hormones" on susceptibility and resistance to tumors Estrogens have already been studied considerably. A good review by Gardner [1] summarizes the results. Gardner, however, was not able to cite any positive results as to the carcinogenic properties of androgens. In the fall of 1935 it occurred to one of us (J. R. M.) that androgens might, if administered in concentrated form, enable the testis to resist the implantation of a tumor. The observation of Murray [2] that "spontaneous" mammary tumors developed in male mice bearing ovarian grafts and the observation of Lacassagne [3] that the same effect could be produced in males by injecting estrogenic substances suggested a possible antagonism between androgens and estrogens as regards carcinogenic effect. Since estrogens had already been proved to increase susceptibility to spontaneous development of tumors, it was surmised that androgens might act in the reverse way and inhibit new growths. With the encouragement and assistance of the department

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From the Department of Vital Economics (Drs. Murlin, Kochakian and Spurr) and the Department of Radiology (Dr. Harvey), University of Rochester.

Dr. S. F. Warren, head of the department of radiology, and Dr. Otto Sahler, formerly of that department, gave advice and assistance throughout this investigation.

1. Gardner, W. U.: Arch. Path. **27**:138-170, 1939.
2. Murray, W. S.: J. Cancer Research **12**:18, 1928.
3. Lacassagne, A.: Compt. rend. Acad. d. sc. **195**:630, 1932.

of radiology, preliminary experiments were therefore begun with the Brown-Pearce epithelioma [4] of the rabbit, with which Dr. Warren and his associate had had considerable experience. The androgenic substances first used were extracted from human urine by the improved method of Kochakian and Murlin.[5] The results when compared with the controls of the department of radiology were sufficiently encouraging that in April 1936 a much larger esperiment was undertaken, and controls inoculated in the same manner and from the same original tumor were carried in parallel with the treated groups. New experiments succeeded as rapidly as space could be provided in the animal house.

Before describing the experiments in detail it may be emphasized that the recent experiments of Murray [6] on parabiosis of male with female mice and the still more recent ones of Nathanson and Andervont [7] on the prevention of spontaneous tumors in female mice by means of testosterone propionate seem to have confirmed the validity of the idea with which we began, although the results in these two investigations concerned only the spontaneous incidence of tumors and not the growth of an implanted neoplasm.

Convinced of a retarding effect on the Brown-Pearce tumor from the use of urinary androgenic extracts, we extended this investigation to several chemically pure androgens in comparison with (1) the urinary extracts, (2) an estrogen and (3) one another; also, particular attention has been paid to the effects of different doses.

STOCK AND CARE

The first five experiments were done on mixed breeds of rabbits obtained from a local dealer. Beginning with experiment 6, all rabbits used have been New Zealand whites, all of which were obtained from the same dealer and assured to be of the same strain. There is good evidence [8] that different strains of rabbits differ sharply in their susceptibility to this tumor. None of the extremely non-susceptible strains were included in any of these experiments. We have had from the start nearly 100 per cent takes, but there has always been a considerable number of spontaneous regressions. These are noted in the tabular and graphic statements (see pages 783 and 784).

Inoculations have been made uniformly by the implanted fragment method, the same tumor supplying all the animals of a given experiment. Pearce and Brown [9] found no essential difference between this method and the emulsion method. Care has been taken to implant a fragment of nearly uniform size (about 15 cu. mm.) in each animal and to insert it with a trocar deep into the testicular parenchyma. The rabbits were young males about 4 months of age, weighing between 2.5 and

4. Brown, W. H., and Pearce, L.: Proc. Soc. Exper. Biol. & Med. **18**:201, 1920.

5. Kochakian, C. D., and Murlin, J. R.: J. Nutrition **10**:437, 1935.

6. Murray, W. S.: Am. J. Cancer **28**:66, 1936.

7. Nathanson, I. T., and Andervont, H. B.: Proc. Soc. Exper. Biol. & Med. **40**:421, 1939.

8. Casey, A. E.: Am. J. Cancer **31**:446, 1937.

9. Pearce, L., and Brown, W. H.: J. Exper. Med. **37**:631, 1923.

3 Kg. They were kept in our own hutches for two weeks before inoculation, and none was used that seemed to be in a poor state of nutrition. The feed for the first five experiments (with the exception of a single group, to be noted) was whole grain oats and alfafa hay. When the change was made to New Zealand whites exclusively, Purina rabbit chow [10] was adopted as possibly a more standard diet. It was found necessary, however, to supplement this with alfalfa hay three times weekly to avoid paralysis. No trouble has been experienced on the latter regime.

From the time of inoculation treated and control animals alike were kept in individual cages and given identical care. In all 306 rabbits were used in the eight experiments recorded from October 1935 to October 1938.

ANDROGENS USED AND THEIR POTENCIES

At the initiation of these experiments there was not a readily available supply of androgens. Consequently it was necessary to prepare them from human male

TABLE 1.—*Potency and Daily Dose of Each Androgen Used*

Experiment	Androgen	Potency		Daily Dose	
		I.U./Mg.	I.U./Cc.	Cc.	I.U.
1A	Urinary extract 67	....	92	0.1*-0.5	9.2-46.0
1B	Urinary extract 567	....	92	0.05*-0.2	4.6-18.4
2	Urinary extract 49	....	112	0.15*, 0.3	16.8, 33.6
	Urinary extract 498	....	80	0.15*, 0.3	12.4, 24.0
	(Estradiol monobenzoate†)	....	500	0.1*, 0.2	50, 100
4§	Urinary extract 498	....	80	0.3	24.0
	Testosterone	50.0	125	0.3	37.5
	(Estradiol monobenzoate*)	....	500	0.3	150.0
5	Urinary extract T.U.	....	625	0.2	125
	Testosterone	50.0	195	0.2	39
6	Urinary extract S-45-1	....	216	0.5 × 2	216
	Urinary extract S-45-2	....	36	0.5 × 2	36
	Testosterone propionate 1	50.0	50	0.5 × 2	50
	Testosterone propionate 2	50.0	10	0.5 × 2	10
7	Androsterone	10.0	50	0.4	20
	Dehydroandrosterone	3.5	17.5	0.4	7
	Testosterone propionate	50.0	250	0.4	100
8	Androsterone	10.0	125	0.4	50
	Dehydroandrosterone	3.5	58	0.3	17.5
	Testosterone propionate	50.0	1,250	0.2	250

* The amount stated is the separate dose for the individual rabbit (see footnotes to table 2).

† The product has the commercial name progynon B. (Schering).

§ Experiment 3 consisted of three control groups.

urine. Later as crystalline products [11] became available commercially, they were introduced. All are listed in table 1 with their potencies as determined in this laboratory by assay with white leghorn capons, following the Gallagher-Koch [12] technic.

The androgens (and the estrogen) were dissolved in oil (olive or sesame, as indicated in table 2) with the aid of ether, which was then removed on the steam

10. The Purina Mills Company has supplied the following analysis of the chow: 2.58 per cent fat, 14.04 per cent protein, 13.44 per cent fiber, 6.04 per cent ash, 7 per cent moisture and 53.03 per cent nitrogen-free extract.

11. Dr. E. Oppenheimer, of Ciba Pharmaceutical Products, and Dr. E. Schwenk, of Schering Corporation, supplied these substances.

12. Gallagher, T. F., and Koch, F. C.: J. Pharmacol. & Exper. Therap. **55**: 97, 1935.

bath. The solutions were sterilized in the autoclave. Injections into tumor-bearing rabbits in most of the experiments were made once daily; in experiment 6, twice daily. Control animals received equal amounts of oil alone. Shortening the interval between injections brings out the full physiologic potency of the androgens, as previously shown,[13] and it would have been desirable, if physically possible, to make more frequent injections.

Urinary extracts as made in this laboratory have the same potency in the rat test per international unit (capon test) as testosterone—in fact, a slightly greater potency. These two preparations therefore, although quite different in chemical composition, may be regarded as of equal physiologic activity with respect to their sex functions. Whether equal or not as regards their influence on carcinogenesis remained to be shown. In experiment 6 the doses of urinary extracts were approximately four times as potent (36: 10 international units; 216: 50 international units, table 1) as the doses of testosterone propionate by the capon test. In this instance, however, as previously reported,[13] the crystalline androgen is four times as active by the rat test as our urinary extracts giving equal capon units. If the effect on carcinogenesis were to be rated according to the influence on growth of the seminal vesicles and prostate rather than according to that on comb growth, one might expect a much greater effect from testosterone propionate. Taking the two tests together we have regarded the urinary androgens in extracts 45-1 and 45-2 as equal physiologically to testosterone propionate preparations 1 and 2 (table 1).

Confirmation as to the greater effect of testosterone propionate than of urinary androgens on the growth of seminal vesicles and prostate was found in the rabbits composing experiments 7 and 8. In experiment 8 these organs were removed at autopsy, soon after injections were stopped, and were weighed. A marked increase in size was observed only in the organs from rabbits which had received testosterone propionate. Those from animals receiving androsterone and dehydroandrosterone showed no increase over those from the control group. It should be emphasized again, however, that the complete physiologic activity is not reflected in the response of the accessory sex glands alone. Dehydroandrosterone, for example, is known to be more efficient in maintaining spermatogenesis than is testosterone.[14]

TREATMENT

As may be seen from tables 1 and 2 a very wide range of doses (in international units) has been employed. In the exploratory experiments (1 and 2) the range was wide even from animal to animal. In the later experiments the dose was kept constant for the entire group of animals throughout the period of injection.

Various times of starting injections as well as various lengths of treatment period were tried. In experiments 1 to 5, inclusive, injections were begun immediately after inoculation and separation of the animals into experimental and control groups. In experiment 6 injections were started five days before inoculation. In experiments 7 and 8 the animals were not divided into groups or treatment started until two weeks after inoculation. At this time the primary tumor had attained a size on which a fair estimate might be made of its condition. The animals thus could be divided more evenly into groups having the same tumor mass. Poor takes or inferior growths were discarded. The periods of

13. Kochakian, C. D.: Endocrinology **22**:181, 1938.

14. Nelson, W. O., in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1937, vol. 5, p. 123.

treatment were always continuous and varied from forty-one to fifty-six days in length. Experiments 1 to 5 were allowed to continue for fifty days more after cessation of injections. Experiment 6 was terminated thirty days after, while experiments 7 and 8 were terminated immediately after, cessation of injection, or approximately sixty days after inoculation. The duration of each experiment from the time of inoculation to the date of the last autopsies is given in table 4.

GROWTH OF PRIMARY TUMOR

The Brown-Pearce tumor implanted in the testis is relatively easy to measure. For a couple of weeks the growth is fairly equal in all diameters; then it begins to conform to the shape of the testis. At this time the two greatest diameters are taken and eventually only the greatest length and the greatest breadth. Occasionally the testis is withdrawn into the pelvis, where the tumor continues to grow, but because it cannot then be pushed through the inguinal canal, it cannot be measured. In such a case the data cannot be included in the study of effects on the primary growth.

The two greatest dimensions are merely added to give a crude measure of the primary mass. It has been found that this mode of expressing mass correlates rather better with the actual weight of the tumor than values obtained by a more correct mathematical procedure, such as calculating the mass of a regular ellipse from its diameters. The tumor mass does not continue long as a geometric figure of any description.

Measurements were made weekly and growth curves plotted. The distribution of sizes so obtained is illustrated in chart 1. In most such curves obtained from our experiments there is a preliminary phase of relatively constant growth rate which has an average duration of about twenty-eight days. This in control animals is followed, as a rule, by a relatively steady state, the size neither increasing nor decreasing greatly for a period which may continue for four to seven weeks.[15] By the end of the first phase metastatic growth usually has begun, and the cessation of rapid growth in the primary tumor doubtless is associated with the latter process. When central necrosis begins, the primary tumor shrinks in size. Sometimes, as in chart 1, this begins so early in individual cases, even in a control group, as to suggest that the tumor has been stifled by the resistance of normal tissue surrounding the implant. Sections of such tumors have been taken but have not yet been studied. Such spontaneous regression in the New Zealand white strain of rabbits in this institution runs to about 30 per cent of all "successful" transplants. This regression rate,

15. It is quite evident from these curves that the growth characteristics of the tumor have changed in the direction of greater regularity since Brown, Pearce and Van Allen (J. Exper. Med. **40**:603, 1924) reported on the first twenty generations of the tumor.

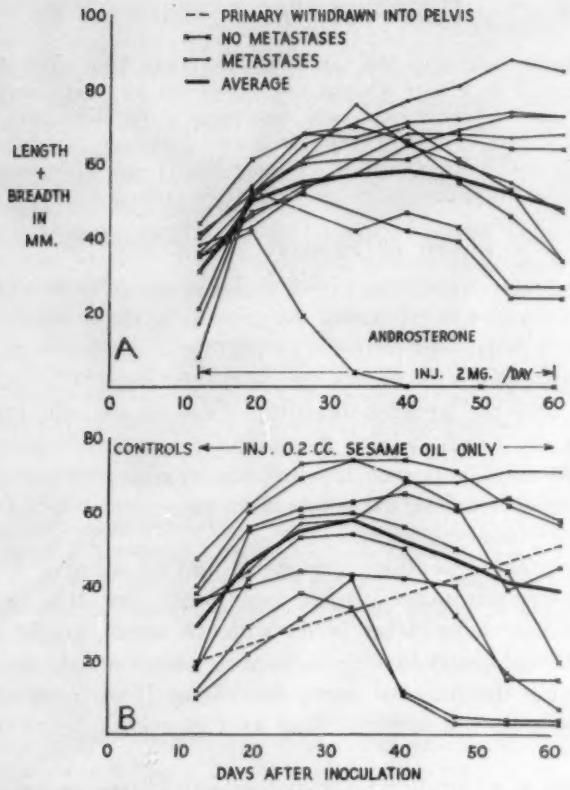

Chart 1.—*A*, rates of growth of primary tumors in experiment 7 with injection of 2 mg. of androsterone a day. *B* (control), rates of growth of primary tumors in experiment 7 with injection of 0.2 cc. of sesame oil only. *INJ* is an abbreviation for injections.

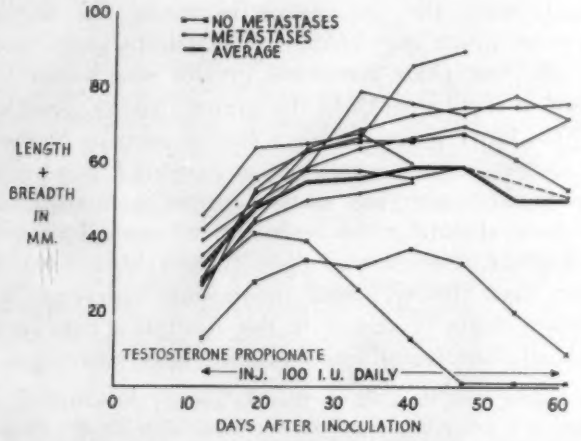

Chart 2.—Rates of growth of primary tumors in experiment with injection of 100 international units of testosterone propionate.

TABLE 2.—*Regression Rate of Primary Tumors*

Experiment	Date	Androgen	Dosage: Dose in International Units	Dosage: Days	Rabbits	Number and Percentage Showing Given Degree of Regression: None No.	None %	Partial No.	Partial %	Complete No.	Complete %
1A	10/ 2/35 2/ 5/36	Urinary extract 67	9.2-46*	48	6	3	50	1	16.7	2	33.3
1B	1/ 7/36 3/10/36	Urinary extract 567	4.6-18.4†	51	4	4	100	0	0.0	0	0.0
2	.....	Urinary extract 49	16.8, 33.6§	50	2	1	50	0	0.0	1	50.0
	4/29/36	Urinary extract 498	12.0, 24.0#	50	8	5	63	2	25.0	1	12.0
	8/ 8/36	(Estradiol monobenzoate)	50, 100‡	50	10	8	80	1	10.0	1	10.0)
		(Olive oil)	.....	50	2	2	100	0	0.0	0	0.0
3	.....	(Sesame oil, injected subcutaneously)	(0.1 cc.)	50	16	12	75	2	12.5	2	12.5
	12/28/36	(Sesame oil, injected into tumor)	(0.1 cc.)	50	15	10	66.7	0	0.0	5	33.3
	3/25/37	(No treatment)	.....	50	12	8	66.7	4	33.3	0	0.0
4	.....	Urinary extract 498	24	50	8	6	75.0	0	0.0	2	25.0
	8/20/36	Testosterone	37.5	50	8	7	87.5	0	0.0	1	12.5
	12/ 9/36	(Estradiol monobenzoate)	150	50	8	5	62.5	2	25.0	1	12.5)
		(Olive oil)	.....	50	9	7	77.8	1	11.1	1	11.1
5	.....	Urinary extract T. U	125	44	13	8	61.5	3	23.1	2	15.4
	4/ 1/37	Testosterone	39	42	14	11	78.6	1	7.1	2	14.3
	7/12/37	(Lettuce)	.....	..	13	10	76.9	0	0.0	3	23.1
		(Control—no oil)	.....	..	13	10	76.9	1	7.7	2	15.4
6	7/15/37	Urinary extract S-45-1	216	56	9	6	66.7	1	11.1	2	22.2
	10/29/37	Urinary extract S-45-2	36	56	9	2	22.3	4	44.4	3	33.3
		Testosterone propionate 1	50	56	10	6	60.0	1	10.0	3	30.0
		Testosterone propionate 2	10	56	10	5	50.0	2	20.0	3	30.0
		(Olive oil)	.....	56	8	4	50.0	3	37.5	1	12.5
7	.....	Androsterone	20	46	11	5	45.5	5	45.5	1	9.1
	3/23/38	Dehydroandrosterone	7	46	11	8	72.7	2	18.2	1	9.1
	5/24/38	Testosterone propionate	100	46	11	8	72.7	1	9.1	2	18.2
		(Sesame oil)	.....	46	11	3	27.3	3	27.3	5	45.4
8	.....	Androsterone	50	41	13	7	53.8	4	30.8	2	15.4
	9/21/38	Dehydroandrosterone	17.5	41	13	9	69.2	3	23.1	1	7.7
	11/19/38	Testosterone propionate	250	41	12	9	75.0	1	8.3	2	16.7
		(Sesame oil)	.....	41	17	11	64.7	2	11.8	4	23.5

* Two rabbits received 9.2, one 18.4, one 27.6, one 36.8 and one 46 international units.
† One rabbit received 4.6, two 9.2 and one 18.4 international units.
§ One rabbit received 16.8 and the other 33.5 international units.
# Four rabbits received 12.0 and four 24.0 international units.
‡ Five rabbits received 50 and five 100 international units.

however, although agreeing with that reported by Brown and Pearce,[16] includes many tumors which do not regress completely within the time of the experiment. Instances may be seen in charts 1 and 2.

The regression rates of the primary tumors in all groups are shown in table 2. The number and percentage of animals showing no regression, partial regression and complete regression are included. The control groups exhibit considerable variations among themselves. It is for this reason obviously that controls must be run with every experiment. In experiment 3, of 15 animals which received injections of sesame oil only into the tumors, one third showed complete regression; two thirds showed none. There were no animals in which regression was partial. Of a group of 16 animals in which oil was injected only under the skin, one eighth (12.5 per cent) showed complete regression and the same percentage partial regression. Among the 12 animals receiving no treatment, not even injections of oil, there were no complete regressions, but 33 per cent showed partial regressions. At first sight there appear to be considerable differences in these three groups, but they concern principally the animals exhibiting partial and complete regressions. It is difficult at times to classify a tumor correctly as between these two categories. Slight traces of tumor may remain, or bits of fibrous tissue may be indistinguishable from tumor to the tactile sense or to the eye. Furthermore, partial regression may be so far advanced that a few days more would place it in the "complete" class. Consequently we regard these three groups as practically equivalent controls. The injection of 0.1 cc. of sesame oil into the tumor has at the most merely hurried up the process of regression slightly, perhaps by pressure necrosis. An even higher regression rate is recorded for experiment 7 in which the daily subcutaneous injection of 0.4 cc. of sesame oil was accompanied by complete regression in 45 per cent of the animals in only sixty-two days. This result, together with the record of no deaths in sixty-two days (table 4), gives evidence that the malignancy of the Brown-Pearce tumor was at this time low, since the susceptibility of the animals should have been high, the time (March) of the year being favorable.[17] Thirty-three per cent complete regressions is the highest rate obtained in any control group (oil only) with the exception just noted.

The range among the control groups is of no great significance because of variations in the animals themselves, possibly because of variations in food and time of year (Brown, Pearce and Van Allen[15]) and certainly because of variations in the malignancy of the

16. Pearce, L., and Brown, W. H.: J. Exper. Med. **37**:799, 1923.
17. Brown, W. H.: Arch. Int. Med. **44**:625, 1929.

tumor as well as in the length of the experiment. Nevertheless it is of interest to find the range extending only from zero to 15.4 per cent for complete regression, zero to 37.5 per cent for partial and 50 to 100 per cent for no regression in the four experiments 2, 4, 5 and 6 which ran to one hundred days or more. How to reduce these variations is now one of our chief problems. Close inbreeding of the experimental stock is well under way, and an improved ration containing all the known vitamins has been adopted. The recent change of rabbit feed, made since experiment 8 was terminated, was suggested in part by the effect of a change of lettuce noted in experiment 5 (chart 3). As the hay and oats ration did not seem to provide a sufficiency of vitamin A, lettuce was introduced with one group of controls. At arrow *A* in chart 3, owing to the fact that Imperial Valley lettuce could not be secured

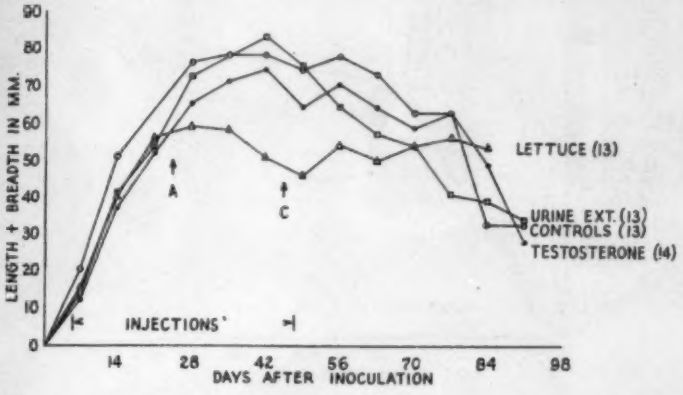

Chart 3.—Average rates of growth of primary tumors in experiment 5.

in the local market, lettuce from a different source but of the same variety was substituted. The average rate of growth of the tumor in this particular group dropped off. Three weeks later (arrow *C*), the original lettuce once more being available, it was restored to the diet, and the growth rate now started upward again. The second lettuce was received in a badly "burned" condition and was wholly unfit for table use. Our thought is that vitamin E contained in such lettuce might have been changed in the process of spoilage so as to become anticarcinogenic. Urinary androgens, it will be noted, hastened regression in this experiment in advance of that in the controls from the seventh week onward.

Long Period Experiments.—There is rather clear evidence in these experiments that treatment with androgens increased the rate of regression. Of the four experiments, 2, 4, 5 and 6, the last gave the best evidence. Fifty per cent of the controls had no regressions; 37.5 per cent had partial and 12.5 per cent complete regressions. The greatest

difference from this distribution occurred in the 9 animals which received 36 international units daily of urinary androgens in extract 45-2. Only 2 animals (22.3 per cent instead of 50 per cent) had no regressions and 7 (77.7 per cent instead of 50 per cent) had partial or complete regressions. The number of animals is too small and the variations are too large to make sure by statistical analysis that this difference is highly significant. We believe it is because of the consistency shown in regression rate with growth rate (chart 4) and with metastases (chart 5).

Regressions obviously are related to the average growth rate of the primary tumor, and in chart 6, bringing together data from the first six experiments, the two groups which have shown the greatest difference from the controls as regards increase in the number and percentage of regressions (table 2) also show a plainly lower average rate of

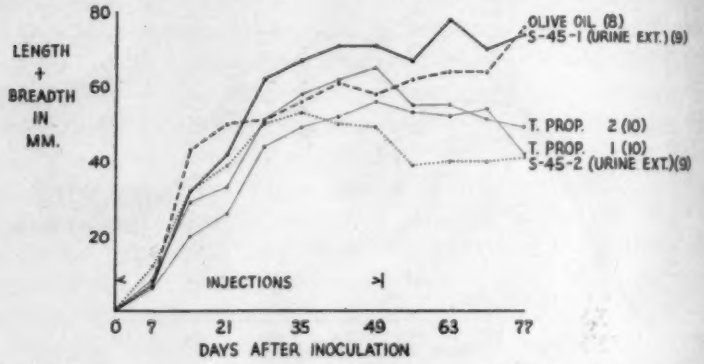

Chart 4.—Average rates of growth of primary tumors in experiment 6 with injection of urinary androgenic extracts and testosterone propionate. The numbers of animals in the control and in the test groups are given in parentheses.

growth than the controls. The one group in experiment 6 showing a definite trend in the opposite direction as regards regressions also shows a higher rate of growth of the primary than the controls (chart 4, *S-45-1*). It is of interest that this group receiving 216 international units of urinary androgens daily produced greater growth and fewer regressions than the controls, while the group receiving only one-sixth as much, i. e., 36 international units daily, of the same androgens, produced less growth and more regressions than the controls. A small dose discouraged the tumor; a large dose enhanced its growth. The difference is not manifest in the two groups which received testosterone propionate in similar proportion (but see page 780).

An estrogen, estradiol monobenzoate, was used in experiments 2 and 4, in comparison with urinary androgens in one and with testosterone and urinary androgens in the other. The results with respect to

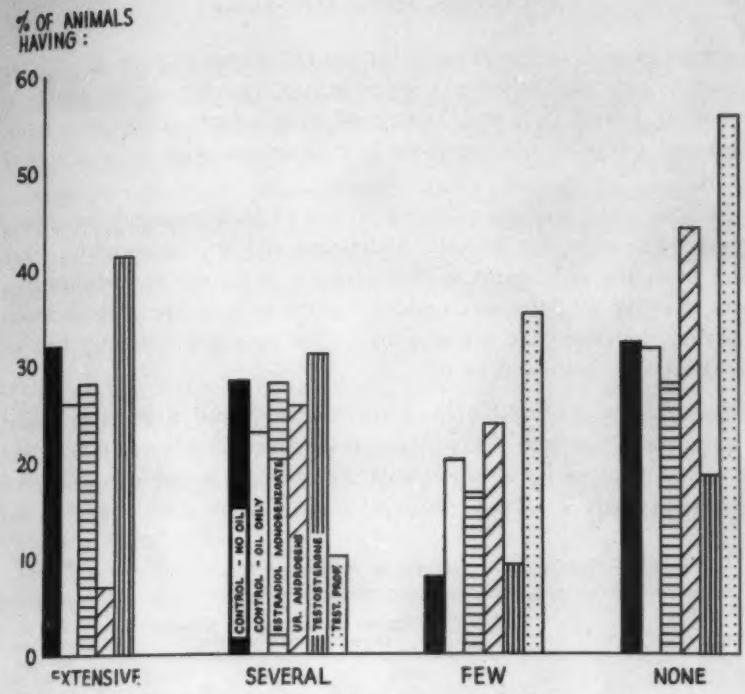

Chart 5.—Effect of androgens (and one estrogen) on the spread of metastases in experiments 1A, 2, 3, 4, 5 and 6.

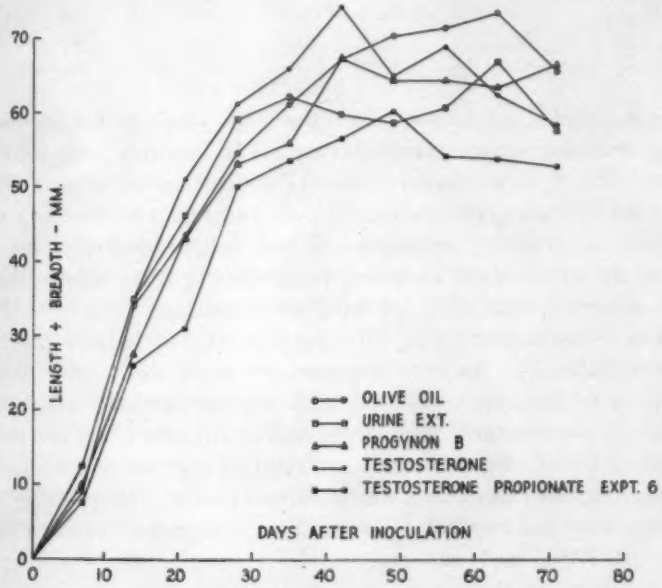

Chart 6.—Average rates of growth of primary tumors in experiments 1A, 2, 3, 4, 5 and 6 (summary).

the average growth of the primary tumors are shown in table 2. A brief summary is provided in table 3 which brings together all the results in experiments 1 A, 2, 3, 4 and 5 obtained with urinary androgens, testosterone and estradiol monobenzoate in comparison with oil as a control injection.

The androgens and the estrogen appear to have changed the regression rate, but only the urinary androgens did so significantly. The change from the oil control in this group is a 12 per cent decrease in tumors showing no regressions and a 9 per cent increase in those showing partial and complete regressions. The estrogen certainly had no more effect than testosterone.

Short Period Experiments.—Experiments 7 and 8 were continued for only sixty-two and sixty days, respectively. The results in the latter experiment so far as growth of the primary tumor was concerned are given in chart 7. Those in experiment 7 were similar except that

TABLE 3.—*Summary of Results in Experiments 1A, 2, 3, 4 and 5*

	Rabbits	Number and Percentage Showing Regression of Given Degree						Sum of Percentages Showing Partial and Complete Regression
		None		Partial		Complete		
		No.	%	No.	%	No.	%	
Urinary androgenic extracts..	41	27	65.9	6	14.6	8	19.5	34.1
Testosterone.....	22	16	72.7	2	9.1	4	18.2	27.3
Estradiol monobenzoate.....	18	13	72.2	3	16.6	2	11.2	27.8
Olive oil.....	40	31	77.5	4	12.9	5	12.5	25.4
Total.....	121							

there was decided regression after the fifth week in all groups save the one receiving dehydroandrosterone The controls regressed most. In experiment 8, in comparison with 17 controls given sesame oil only, none of the animals given chemically pure androgens showed any significant effect on growth. Androsterone and dehydroandrosterone, which occur in the urine, were expected to produce greater effects than the urinary extracts containing an equivalent unitage. But this did not take place (charts 4 and 7). The same is true as regards the regression rate (table 2). In both experiments there were fewer complete regressions in the groups treated with androgens than in the control groups. In experiment 7 there were only 3 animals (27.3 per cent) of the control group which showed no regressions, but in experiment 8 there were 11 (64.7 per cent) which showed none. This probably means that the tumor had reached a low state of malignancy in experiment 7 (spring of 1938) and was restored by frequent animal passage to a higher virulence in experiment 8 (fall of 1938).

The greater effect of urinary androgenic extracts on primary growth and regressions than of chemically pure products suggests that there may

be some as yet little known or unrecognized steroid substance in human urine, extracted with the same procedure as the androgenic substances, which is responsible for the effect, or that the chemically pure androgens in the concentrations used are too strong in their physiologic actions when taken separately, whereas the urinary extracts containing several different chemical entities have an effect on carcinogenesis that is the result of some sort of combined action. One hormone may to an appreciable extent counteract another (Kochakian [18]).

METASTASIS

This tumor has a rather characteristic or preferred pattern of distribution, according to Casey.[19] However, we have not much faith in statistics applied merely to the occurrence of nodules in different loci. In another investigation (unpublished), begun considerably later than

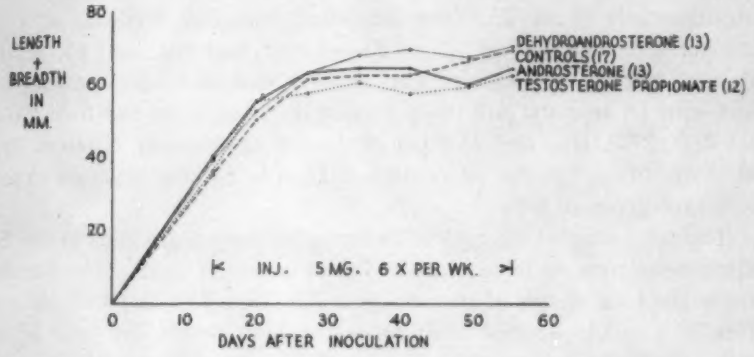

Chart 7.—Average rates of growth of primary tumors in experiment 8 (summary). The numbers of controls and of animals in the groups given androgens are given in parentheses.

this one, we have undertaken a study of the distribution of the metastatic nodules not only topographically but also quantitatively by weighing all the nodules. For the present report we have been obliged to content ourselves with division into four classes of metastasis: (1) extensive, i. e., diffuse involvement of six or more sites; (2) several, three to six sites with multiple nodules; (3) few, i. e., less than four scattered small metastases; (4) none. The distribution into these classes is shown in table 4. Also shown are the number and percentage of "extensive" and "several" metastatic growths collectively in columns parallel with the mortality rate.

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Because metastasis once started is likely to continue spreading farther and farther the longer the animal is permitted to live, unless by chance it strikes a vital organ early, it is necessary to consider separately the experiments which continued one hundred days or longer and those which continued only sixty days approximately. Experiments 1A, 2, 4, 5 and 6 fall into the former class; experiments 1B, 7 and 8, into the latter. Experiment 7 at least is exceptional, as we have shown, because the tumor, as judged by several criteria, was in a somewhat lower state of malignancy.

Long Period Experiments.—There are two kinds of controls to be considered first. In experiments 3 and 5 there were, all told, 25 rabbits which received no treatment, not even injections of oil. Of these, 8, or 32 per cent, had extensive metastases; 7, or 28 per cent, had several, 2, or 8 per cent, had few, and 8, or 32 per cent, had none. In fair agreement with these figures, among the 35 controls receiving oil only subcutaneously 9, or 25.8 per cent, had extensive metastases; 8, or 22.8 per cent, had several; 7, or 20 per cent, had few, and 11, or 31.4 per cent, had no metastases. The estrogen estradiol monobenzoate was used with 18 animals, and the percental distribution to the four classes was 27.8, 27.8, 16.6 and 27.8 per cent—not significantly different from the distribution for the oil controls. Details for the separate experiments are given in table 4.

The total number of rabbits receiving urinary androgens in the five experiments running to one hundred days or longer is 55. The distribution to the four classes of metastases is 7.3, 25.4, 23.6 and 43.6 per cent. Here is a rather decided shift from the high to the low side of the scale. Testosterone, on the other hand, given to 22 animals, gave the distribution 40.9, 31.8, 9.1 and 18.2 per cent—a decided shift to the high side. Testosterone propionate, however, gave an even greater shift to the low side than did the urinary androgens; namely, 0, 10, 35 and 55 per cent for the four classes.

A summary of these six different kinds of tests in which the experiments ran to one hundred days or more is given in table 5.

Short Period Experiments.—Only 4 animals having been used in experiment 1B and all of them having received urinary androgenic extracts, this group is omitted in the consideration of the crystalline androgens. It happened also that this small group gave an altogether exceptional result in comparison with any other group receiving androgens with respect both to growth and to regressions, on the one hand, and with respect to metastases, on the other, especially so when the short duration of the experiment is considered.

Experiments 7 and 8 were identical in all respects except the strength of doses used (table 4). In an earlier section attention was

TABLE 4.—*Comparison of Severity of Malignant Growth and Mortality as Affected by Different Androgens*

Duration of Experiment, Days	Experiment	Androgen	Dosage*		Rabbits	Number and Percentage Showing Given Degree of Metastasis										Number and Percentage Dying†	
			Dose in International Units	Days		Extensive		Several		Few		None		Extensive + Several			
						No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
136	1A	Urinary extract 67	9.2-46.0	48	6	1	16.7	2	33.3	0	0.0	3	50.0	3	50.0	3	50.0
62	1B	Urinary extract 567 (smaller dose)	4.6-18.4	51	4	2	50.0	2	50.0	0	0.0	0	0.0	4	100.0	4	100.0
102	2	Urinary extract 49	16.8, 33.6	50	2	0	0.0	0	0.0	0	0.0	2	100.0	0	0.0	0	0.0
		Urinary extract 498	12.0, 24.0	50	8	2	25.0	1	12.5	1	12.5	4	50.0	3	37.5	4	50.0
		Estradiol monobenzoate	50, 100	50	10	4	40.0	2	20.0	2	20.0	2	20.0	6	60.0	8	80.0
		(Olive oil)	.....	50	2	1	50.0	0	0.0	1	50.0	0	0.0	1	50.0	2	100.0
87	3	(Sesame oil, injected subcutaneously)	(0.1 cc.)	50	16	7	43.8	5	31.2	0	0.0	4	25.0	12	75.0	10	63.0
		(Sesame oil, injected into tumor)	(0.1 cc.)	50	15	7	46.7	2	13.3	2	13.3	4	26.7	9	60.0	9	60.0
		(No treatment)	.....	50	12	6	50.0	0	0.0	1	8.3	5	41.7	6	50.0	6	50.0
112	4	Urinary extract 498	24	50	8	0	0.0	1	12.5	4	50.0	3	37.5	1	12.5	4	50.0
		Testosterone	37.5	50	8	4	50.0	2	25.0	1	12.5	1	12.5	6	75.0	7	87.5
		Estradiol monobenzoate	150	50	8	1	12.5	3	37.5	1	12.5	3	37.5	4	50.0	5	62.5
		(Olive oil)	.....	50	9	1	11.1	1	11.1	5	55.6	2	22.2	2	22.2	5	55.6
103	5	Urinary extract T. U	125	44	13	1	7.7	6	46.2	3	23.1	3	23.1	7	53.9	7	53.9
		Testosterone	39	42	14	5	35.7	5	35.7	1	7.1	4	21.4	10	71.4	10	71.4
		(Lettuce)	.....	..	13	3	23.1	7	53.9	0	0.0	3	23.1	10	77.0	10	77.0
		(Control—no oil)	.....	..	13	2	15.4	7	53.9	1	7.7	3	23.1	9	69.2	10	77.0
106	6	Urinary extract S-45-1	216§	56	9	0	0.0	4	44.4	1	11.1	4	44.4	4	44.4	5	55.5
		Urinary extract S-45-2	36§	56	9	0	0.0	0	0.0	4	44.4	5	55.6	0	0.0	1	11.1
		Testosterone propionate 1	50§	56	10	0	0.0	2	20.0	3	30.0	5	50.0	2	20.0	3	30.0
		Testosterone propionate 2	10§	56	10	0	0.0	0	0.0	4	36.3	6	63.7	0	0.0	2	20.0
		(Olive oil)	.....	56	8	0	0.0	2	25.0	1	12.5	5	62.5	2	25.0	2	25.0
62	7	Androsterone	20	46	11	2	18.2	0	0.0	3	27.3	6	54.6	2	18.2	1	9.1
		Dehydroandrosterone	7	46	11	2	18.2	1	9.1	4	36.4	4	36.4	3	27.3	2	18.2
		Testosterone propionate	100	46	11	3	27.3	3	27.3	2	18.2	3	27.3	6	54.6	3	27.3
		(Sesame oil)	.....	46	11	1	9.1	0	0.0	0	0.0	10	90.9	1	9.1	0	0.0
60	8	Androsterone	50	41	13	1	7.7	2	15.5	6	46.6	4	31.0	3	23.3	1	7.7
		Dehydroandrosterone	17.5	41	13	1	7.7	2	15.5	2	15.5	8	61.5	3	23.3	0	0.0
		Testosterone propionate	250	41	12	1	8.3	1	8.3	5	41.7	5	41.7	2	16.6	1	8.3
		(Sesame oil)	.....	41	17	3	17.7	2	11.8	7	41.2	5	29.4	5	29.4	1	5.0

* See footnotes to table 2 for more details.

§ See page in text concerning relative potencies of these substances in the rat test.

† Usually when the percentage of rabbits dying is greater than the percentage of those showing "extensive plus several metastases," the difference is accounted for by the fact that some of those having few nodules had those nodules in a vital position, e. g., a kidney, a lung, the neck of the bladder or the spinal cord (paralysis).

arrested by the difference in effect on growth between a large and a small dose of urinary androgens in experiment 6. This is barely visible in the metastases (table 4, experiment 6). The same is true as between the two levels of testosterone propionate given in the same experiment. No great point can be made of a shift from several to few metastatic nodules.

This being true of an experiment which ran to one hundred and six days from midsummer (when rabbits appear to be more resistant than in any other season [17]), perhaps it was too much to expect any difference between doses of different size in a short experiment of only sixty-two days in early spring and fall. At all events there is no significant

TABLE 5.—*Long Period Experiments*

	Rabbits	Number and Percentage Showing Given Degree of Metastasis							
		Extensive		Several		Few		None	
		No.	%	No.	%	No.	%	No.	%
Control, no oil	25	8	32.0	7	28.0	2	8.0	8	32.0
Control, oil only	35	9	25.8	8	22.8	7	20.0	11	31.4
Estradiol monobenzoate	18	5	27.8	5	27.8	3	16.6	5	27.8
Urinary androgenic extracts	55	4	7.3 (−21)*	14	25.4 (−1)	13	23.6 (+10)	24	43.6 (+12)
Testosterone	22	9	40.9 (+12)	7	31.8 (+5)	2	9.1 (−5)	4	18.2 (−14)
Testosterone propionate	20	0	0.0	2	10.0	7	35.0	11	55.0
Total	175		(−29)		(−16)		(+21)		(+23)

* The figures in parentheses represent the changes in percentage from an average of the two control groups. The shift to the low side of the scale of metastasis caused by urinary androgens and by testosterone propionate is impressive. The opposite effect of testosterone is not so great.

difference between 20 and 50 international units of androsterone in the two experiments, only a slight indication of difference between 7 and 17.5 international units of dehydroandrosterone and differences of doubtful import between 100 and 250 international units of testosterone propionate. The differences from controls shown by one group in few and no metastases cancel each other and the differences in extensive and several metastases do likewise. There is no general shift to the low side or the high side of the scale, such as was seen with the urinary androgens and testosterone propionate in smaller doses. The two control groups themselves differed greatly, owing principally, we believe, to the short period of the entire experiment.

Putting together the two experiments, we get the summary of results given in table 6. This table brings out the fact that there is no clearly

significant change from the control group in either direction which could be attributed to any of the crystalline androgens. The nearest approach to it is again with testosterone propionate, which has caused a moderate shift to the high side; i. e., in the opposite direction to that observed in the long period experiments with much smaller doses. Quite possibly, if these experiments had been continued to one hundred days, this would have become a pronounced shift, and the difference between small and large doses would have been illustrated even more sharply than in the growth effects of the urinary androgens.

TIME OF STARTING INJECTIONS

As noted in the section on treatment, in experiments 1 to 5 inclusive, the injections were begun at the time of inoculation; in experiment 6, five days before inoculation, and in the last two experiments, two

TABLE 6.—*Short Period Experiments*

		Number and Percentage Showing Given Degree of Metastasis							
		Extensive		Several		Few		None	
	Rabbits	No.	%	No.	%	No.	%	No.	%
Controls	28	4	14.3	2	7.1	7	24.6	15	53.5
Androsterone	24	3	12.5	2	8.2	9	37.5	10	41.0
Dehydroandrosterone	24	3	12.5	3	12.5	6	25.0	12	50.0
Testosterone propionate	23	4	17.3	4	17.3	7	30.4	8	34.6
			+3		+10		+6		−18.9

weeks after inoculation. Careful scrutiny of tables 2 and 4 reveals no essential difference in results except in the one experiment in which injections were started previous to inoculation. The greatest effect of urinary androgens and testosterone propionate on growth, the greatest difference between a large dose and a small dose, the most consistent difference from their own controls as regards the effect of both urinary androgens and testosterone propionate on regression rate, and certainly one of the most significant general effects on metastasis (see table 5) occurred in this experiment. No animal in the entire experiment had the fulminating type of metastasis seen in at least several animals in every other experiment.

It appears therefore that injection of androgens previous to inoculation influenced the suitability of the rabbit tissues in such a way as to produce a less favorable soil for this tumor. It is true that none of the 8 controls showed extensive metastases, but at least one other control group (experiment 4) of a comparable number of animals and running an even longer period had only one animal with the extensive grade, and the distribution to the other grades of metastasis in this experiment

is not out of line with that in the others. The peculiar result is not so much that the control group numbering 8 animals did not show a single one with this degree of severe metastasis, as that none of the treated groups, numbering 38 animals all told, had such an animal. We have no explanation other than the effect of injections started before inoculation. The seasonable decrease in susceptibility should not have affected this experiment more than experiments 4 and 8. The signs of diminished malignancy, fewer extensive metastases and lower mortality rate were only a little greater than in any other control group.

It is of significance that urinary androgens and testosterone propionate, the only ones used in experiment 6, gave evidence of effects on the tumor in other groups as well.

In the last two columns of table 4 are shown the number and percentage of animals with extensive and several metastases collectively side by side with the number and percentage of the animals in each group which died of carcinomatosis, and nothing else, within the period of the experiment. Of the thirty groups of animals listed, containing 306 animals, nine groups, containing 87 animals, or 29 per cent of the entire number, exhibit the same percentages in the two columns. Severe metastasis runs exactly parallel with mortality. Twelve groups, containing 126 animals, differ from each other in the two columns not more than 12.5 per cent, the average being 10.3 per cent. The remaining nine groups, containing 93 animals, differ by an average figure of 27.8 per cent. It is obvious that the severe grade of metastasis, as we have classified it, is a primary cause of death.

The highest mortality percentages naturally occur in the long period experiments and many of the lowest in the short period ones. However, there are many low figures for mortality, as we have observed is true for severe metastasis, in the long term groups that are treated with androgens, particularly urinary androgens and testosterone propionate (table 4, experiments 1A, 2 and 6). The estrogen in experiment 2 and testosterone in experiments 4 and 5 produced high figures. In experiment 4 the figure is notably higher than that for the corresponding control group; in experiment 5 it is a little lower. In experiment 4 the estrogen produced figures for mortality closely similar to those of the controls, but in experiment 2 the number of control animals was too small for a satisfactory comparison. The average percentage of animals in all of the twenty-one long period groups, control and experimental, showing severe metastasis is 47.7; the average mortality rate is 51.6. The total number of animals involved is 203. The averages for the 75 controls alone are 50.3 per cent for severe metastasis and 61.5 per cent for mortality. The corresponding figures for the 55 animals getting urinary androgens are 26.9 and 38.6.

COMMENT

The chemical nature of the androgens and estrogens is now so well known or is described in so many publications [20] that it need not be discussed except as it may conceivably have affected the results of these experiments. Being steroids, these substances are fat soluble and, it may be presumed, penetrate the cells through the lipoid component of protoplasm. They are not known to be phosphorylated in any way and therefore probably exert their influence on cell metabolism independently of phospholipids. They may constitute an entirely new enzyme system, for it is evident from the chemistry of their artificial production and the dependence of their potency on the number and position of their OH and O groups that they are reducing and oxidizing substances.

Androgens, however, have little effect on the intensity of oxidation in the body as a whole.[21] On the other hand, their role seems not to be limited to the highly specialized tissues contained in the sex organs; for they produce a better nutritive condition, as indicated by body weight, when administered in presumably physiologic amounts to castrate dogs [5] or to eunuchoid men.[21c] Excessive doses, however, definitely decrease the growth rate of male but not that of female mice.[18] Intermediate between these effects are those of McEwen, Selye and Collip,[22] who have shown that growth of young rats is not retarded by a daily dose of testosterone which inhibited gonad development. Dosage, therefore, is important.

The doses used in these experiments were favorable for general nutrition and also for the effects on the accessory sex glands (see page 780). In experiments 7 and 8, in which body weights were recorded weekly, the largest doses of testosterone propionate were used. Yet the growth in weight was decidedly superior to that of the controls. No estimates were made of effects on muscular development, but it is altogether likely that this was accelerated as reported in the experiments of Papanicolaou and Falk [23] for guinea pigs. The deposition of protein then in general as well as in specialized tissues was augmented. The use of toxic doses would probably have interfered with normal anabolism.

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Can the "sex hormones" be related definitely to pathologic anabolism as in neoplasms, and, if so, how do they bring about such effects? There is some pertinent evidence as to this in the cases of virilism. A tumor in the adrenal cortex is accompanied by excessive excretion of androgens in the urine, chief of which is dehydroandrosterone,[24] normally occurring only in very small amounts. The excess of androgens in the urine promptly decreases on removal of the tumor.[24a] The occurrence of an excess of androgens together with the abnormality of virilism suggests the former (while in the body) as the cause of the latter. There occur also in such urines other apparently normal compounds of a steroid nature [25] both of the pregnene-pregnane and of the androstene-androstane series. Their presence indicates an increased and altered metabolism of normally occurring substances. It is conceivable that these steroids closely related to androgens and estrogens play an active part in the heightened cellular metabolism of the rapidly growing carcinoma and that their altered metabolism is a contributing factor in the pathologic anabolism. That they operate by virtue of their oxidation and reduction properties is indicated, as we have pointed out, by the universal occurrence in steroid structures of oxidizing and reducing groups. Furthermore, they occur in the generating tissues not as a single substance but as a group with markedly varying degrees of physiologic activity as shown by the state of oxidation and steric configuration of the molecule. In the adrenal cortex, in particular, the number of such isomers or epimers of varying degrees of oxidation and reduction is relatively enormous. Similar compounds occur in the urine,[26] but none of them possesses physiologic activity; they have completely run down.

Steroid substances, then, inclusive of "sex hormones," are either reduced or oxidized in the course of their physiologic duties and the resulting product or products have much less activity than the original substances. In the case of the pregnene-pregnane group (those from adrenal cortex or corpus luteum) the substances excreted in the urine have no physiologic activity so far as determined by known methods. For example, it has been shown [27] that after injection of progesterone there is excreted a comparable amount of pregnandiol glycuronate, which is a completely reduced form of progesterone. The same product occurs during corpus luteum activity in pregnancy, and, in addition, other reduced compounds occur both in the corpus luteum and in the urine.

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The products elaborated by the testis have not been extensively studied. It has been demonstrated, however, that more than one steroid substance is present and that testosterone is probably the most active one turned out. In the urine are found androsterone, dehydroandrosterone, probably isoandrosterone and epietocholandiol. Dehydroandrosterone is of particular interest in connection with the present study. It contains all the polar groupings of testosterone, yet is decidedly inferior to the latter in its physiologic activity. The difference may be explained by the position of the polar groups. Hydroxyl in testosterone is in the 17 position, while in dehydroandrosterone it is in the 3 position. The carbonyl in testosterone is in the 3 position, forming an $\alpha\ \beta$ unsaturated grouping, which apparently is favorable for physiologic activity, probably by reduction. This grouping is possessed also by corticosterone and progesterone. Dehydroandrosterone has its carbonyl in the 17 position. The reversal in the position of these two groupings in dehydroandrosterone as compared with testosterone is taken as evidence that the former originates from the latter by stepwise or simultaneous oxidation and reduction which results in a much diminished physiologic activity. Possibly it is the order of these two reactions which is important.

It is significant, we think, that of all the androgens, the less active, by the usual methods of assay, are the ones most reduced. They seem to be the least effective also in checking the growth of the Brown-Pearce tumor, though our data are far from complete. Certainly the most active both on secondary sex organs and on the tumor is testosterone propionate. The ester form is believed to augment the activity only by retarding the absorption rate and protecting the hydroxyl group and thereby economizing the use of the material [28]; but it may, in some way not yet understood, facilitate the supposed uptake and exchange of hydrogen, thus making testosterone a more speedy oxidizing agent.

In contrast the estrogens exhibit the converse relationship—the more reduced the form the greater the activity. Estradiol (dihydrotheelin), the form occurring in the ovarian follicle, is at once more reduced and more active than estrone (theelin). The former has hydroxyl, the latter carbonyl, in the 17 position. Furthermore, the hydrated form, estriol (theelol), with hydroxyls in the 16 and 17 positions, is less active than the alcoholic estradiol, probably because water is so easily given off, forming the carbonyl again.

This peculiarity of estrogens is of particular significance, since of all the steroid hormones only the estrogens have to date been proved to have carcinogenic properties. The androgens in certain instances, as we

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have shown in this study, and in the case of spontaneous neoplasms, are able to retard or prevent the induction of tumor development. It is our hypothesis, therefore, that estrogens owe their carcinogenic activity to the necessity for their oxidation; i. e., they tend to rob cells of O_2 and leave them dependent on anaerobic glycolysis. Prevention of this action is accomplished by androgens, which provide hydrogen acceptors, thereby adequately titrating the hydrogen which estrogens have to spare and thus defeating the attack on normal cell life.

SUMMARY

A total of 306 rabbits inoculated in one testis with a fragment of Brown-Pearce epithelioma were treated with the following androgens in groups nearly equal in numbers to the control groups: Urinary androgenic extracts, 4.6 to 216 international units; androsterone, 20 and 50 international units; dehydroandrosterone, 7 and 17.5 international units; testosterone, 37.5 and 39 international units; and testosterone propionate, 15, 50, 100 and 250 international units; also estradiol monobenzoate, 50, 100 and 150 international units. Injections were made daily for forty-one to fifty-six days beginning, in different experiments (*a*) five days before (*b*) at time of and (*c*) fourteen days after inoculation. The experiments were terminated variously, i. e., immediately and thirty and fifty days after cessation of injections.

The urinary androgenic extracts, especially in the smaller doses, caused increased regression of the primary tumors. The same effect, however, could not be produced with androsterone or dehydroandrosterone, the known androgens of the urinary extracts. It is suggested that this result may be due, to some other steroid (or steroids) or a combination of substances in the extract. In the experiments continued after cessation of injections the urinary extracts and testosterone propionate decreased, testosterone increased and estradiol monobenzoate, sesame oil and olive oil left unaffected, the amount of metastasis. In the short period experiments, however, androsterone, dehydroandrosterone and also testosterone propionate showed no or very slight effect.

The greatest differences were noted when injections were begun prior to inoculation of the tumor.

Taking into account all experiments with urinary androgens, the reduction in severe metastasis for 55 animals in comparison with 75 controls was from 50.3 to 26.9 per cent, and that in mortality was from 61.5 to 38.6 per cent, a strictly parallel effect.

A hypothesis is proposed to account for the opposite carcinogenic effects of androgens and estrogens.

ASPIRATION TYPE OF CONGENITAL TUBERCULOSIS

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After decades of controversy, congenital tuberculosis has become a recognized entity. Excellent reviews of the subject can be found in the articles of Hamne and Gellerstedt,[1] Siegel[2] and Giuliani.[3] Although there are at least four forms—(1) the more common hepatic, (2) the generalized miliary, (3) the ingestion and (4) the aspiration type—the present article is concerned only with the fourth.

The painstaking researches of Schmorl and Geipel,[4] who collected 42 cases, demonstrated the existence of placental tuberculosis. Tuberculous nodules were found in the chorion outside of the placenta as well as elsewhere, and so tubercle bacilli might invade the liquor amnii. Several authors have demonstrated the bacilli in the liquor,[1] and Geipel even found sheaves of organisms, pointing to a growth of bacilli in the fluid.[1] It is now recognized that respiratory movements occur in the fetus in utero.[5] The conditions which theoretic consideration requires for the development of the aspiration type of congenital tuberculosis are thus established.

The first mention of the aspiration type which we found in the literature is that by Andrewes.[6] Unfortunately, the description of the case is so meager that the diagnosis cannot be accepted without reservation. Much of the older literature is rendered equivocal because of the same fact; either the isolation of the child after birth is not absolutely assured or the autopsy is in one way or another incomplete. Stains for the organism or cultures are frequently lacking. In 1913

From the Department of Pathology of Cleveland City Hospital and the School of Medicine of Western Reserve University.

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2. Siegel, M.: Am. Rev. Tuberc. **29**:297, 1934.
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4. Schmorl, G., and Geipel, P.: München. med. Wchnschr. **51**:1676, 1904.
5. Snyder, F. F., and Rosenfeld, M.: Proc. Soc. Exper. Biol. & Med. **36**:45, 1937; Am. J. Obst. & Gynec. **36**:363, 1938.
6. Andrewes, F. W.: Tr. Path. Soc. London **54**:142, 1902-1903.

Harbitz [7] reported a case with sufficient evidence to render the diagnosis certain. However, the author, although realizing the fact that he was dealing with congenital tuberculosis, did not recognize the type. An excellent review of this particular phase of the subject was published in 1934 by Giuliani.[8] The accompanying table contains a résumé of the cases we have collected after a critical review of the literature. Of the authors, only 7, Kraus,[8] Siegel,[9] Harbitz,[7] Couvelaire and others,[10] Zarfl,[11] Giuliani [8] and Gander,[12] described cases which can be accepted without reservations. Brindeau,[13] Orefice [14] and Andrewes [6] did not furnish the microscopic descriptions necessary to exclude miliary tuberculosis by way of the umbilical vein and ductus venosus Arantii. Furthermore, there is no certainty that the child was removed from all possible tuberculous contagion immediately after birth. In this respect, however, the time of death (twelfth and sixteenth day and "shortly after birth") appears to render a postpartum infection highly improbable. Just this point, on the other hand, makes the case of Delmas [15] unacceptable; until further evidence is presented, it is improbable that a child infected before or at birth could live to the age of 4 months. The fact that no microscopic examination was made of any organ except the lung is a further reason for eliminating this case. In the case of Trillat and others [16] the description of the lungs is strongly suggestive of a typical postpartum primary complex. There are several others of the same type. These differ so radically from the unquestionably authentic cases mentioned that they have not been accepted by us. The question whether they represent postpartum aerogenous infections or congenital infections by way of the ductus Arantii, or whether there is a form of congenital aspiration tuberculosis resembling the postpartum primary complex must be left open for further study.

To the group of well established cases we add a case observed in the pediatric service, with autopsy in the Central Laboratories of Cleveland City Hospital.

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7. Harbitz, F.: München. med. Wchnschr. **60**:741, 1913.
8. Kraus, E.: Centralbl. f. allg. Path. u. path. Anat. **63**:273, 1935.
9. Siegel, M.: Beitr. z. path. Anat. u. z. allg. Path. **90**:503, 1932.
10. Couvelaire, A.; Valtis, S., and La Comme, M.: Bull. Soc. d'obst. et de gynéc. **17**:31, 1928.
11. Zarfl, M.: Ztschr. f. Kinderh. **58**:266, 1936.
12. Gander, G.: Ann. d'anat. path. **9**:891, 1932.
13. Brindeau, M. A.: Bull. Soc. d'obst. de Paris **2**:235, 1899.
14. Orefice, G.: Pediatria **35**:1138, 1927.
15. Delmas, P.: Obstetrique **3**:534, 1910.
16. Trillat, P.; Eparvier; Bertoye, P., and Bertrand: Nourrisson **25**:168, 1937.

REPORT OF A CASE

E. K., a white boy, was born at another hospital on Sept. 23, 1938. His weight at birth was 5 pounds 2 ounces (2,324.5 Gm.). The child was immediately removed from the presence of the mother and was never after in contact with her or any other person known to be tuberculous. The food was milk removed from the mother's breasts by pump. The child was discharged to the care of an aunt on October 1. At that time he weighed 4 pounds 8 ounces (2,041 Gm.) and appeared well. On October 6 diarrhea set in. Anorexia, vomiting and somnolence followed. On his admission to Cleveland City Hospital, on October 9, his weight was 2,075 Gm. He was slightly cyanotic and dehydrated; there were no other physical findings. The urine was normal. The red blood cell count was only 3,331,000

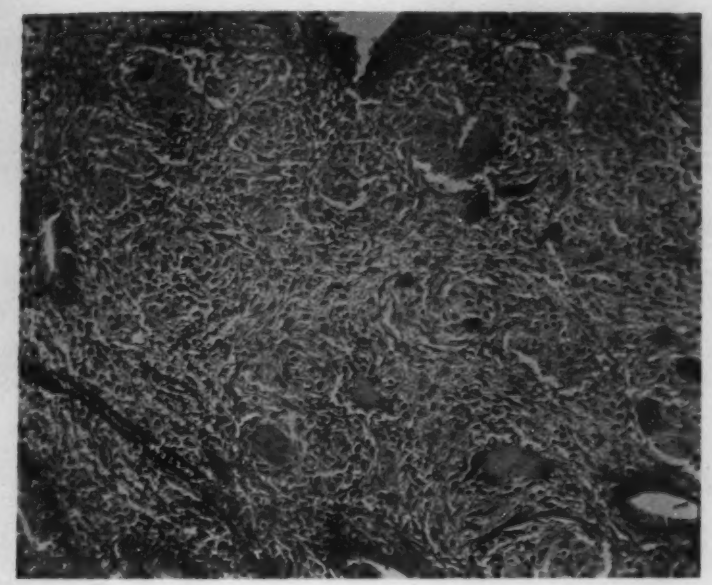

Fig. 1.—Tubercle in postpartum curettings; hematoxylin and eosin; × 126.

per cubic millimeter, the hemoglobin 60 per cent and the white blood cell count 13,600. The intracutaneous tuberculin test (1 : 1,000) was negative. The most striking features were the sudden onset and the rapid course of the disease. The temperature, which had been 37.2 C. (98.9 F.) at admission, rose to 39.5 C. (103.1 F.) and then fell to 37.4 C. (99.3 F.) on the third day of the stay in the hospital and the nineteenth day of life. Aside from increasing cyanosis and rales at the bases of both lungs, nothing else was observed. The clinical diagnosis was bronchopneumonia, prematurity, malnutrition and foreign body in the trachea.

The mother was known to be suffering from tuberculosis and was transferred to Lowman Pavilion, of the Cleveland City Hospital. Unfortunately, no information concerning the placenta could be obtained. However, tissue obtained by curettage on December 2, by Dr. James H. Jewell, of the Department of Obstetrics, Cleveland City Hospital, was grossly hyperplastic and very friable and contained small areas of grayish white material, which appeared to be caseous (fig. 1).

The report of the pathologist was: "The stroma is to a large degree replaced by tubercles, which are of typical structure, showing many epithelioid cells, as well as a moderate number of giant cells of Langhans. In a few cases there is a central area of necrosis with a moderate number of polymorphonuclears. Here and elsewhere the tubercles are rupturing into glandular spaces. The Ziehl-Neelsen stain for acid-fast bacilli is positive. The diagnosis is tuberculous endometritis."

Postmortem Examination.—The autopsy was performed by Dr. M. C. Wheelock, three hours after death. The essential observations were as follows: On removal of the chest plate the lungs completely filled the thoracic cavity. The parietal and visceral pleurae were smooth, gray and glistening. Beneath the pleura were small grayish yellow nodules, 1 mm. or less in diameter (fig. 2). The lungs were firm and sank readily in water. There was no crepitation. Solution of formaldehyde

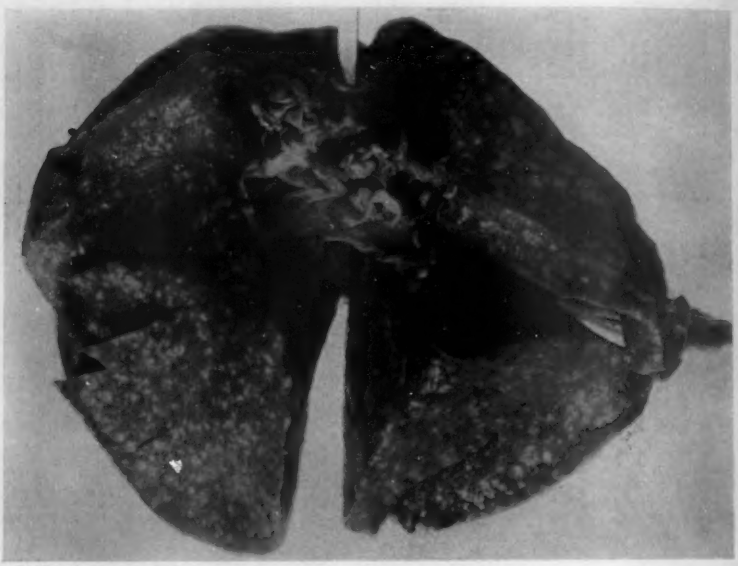

Fig. 2.—Lungs of an infant with congenital pulmonary tuberculosis of the aspiration type.

U. S. P. was injected through the pulmonary artery. Section through the individual lobes of the lungs demonstrated increased resistance. The cut surface was similar to the external one. The smaller grayish yellow nodules were present diffusely throughout all sections. The intervening lung was likewise firm but was brown and gray. There was no enlargement of the mediastinal lymph nodes; they did not measure more than 7 mm. None of the other organs, including the central nervous system, showed any lesions in the gross. In particular, the lymph nodes of the abdomen were not enlarged.

Microscopic Examination.—A coronal section through the midzone of the right lung showed the parenchyma to be diffusely beset with lesions measuring at the most 2 mm., although most of them were 1 mm. or less. The size of these areas did not vary with their position in the lung. There were, however, more of them per square centimeter at the base. Each one consisted of infiltration into a

number of alveoli, the whole being usually circular and rather well demarcated from the surrounding lung; in other words, the condition was a localized and perhaps lobular pneumonia. Higher magnification showed a fibrillar material which stained deep blue with hematoxylin and represented an actual cast of an alveolus. In this material were found cells which were of the reticuloendothelial type. A few polymorphonuclears were seen, but the oxidase stain showed that these comprised at the most 10 per cent of the entire number; in fact, the total number of cells in the affected alveoli was small. Despite the small number of polymorphonuclears it was striking that eosinophils were seen among them. The alveolar walls were for the most part retained. There was little destruction and no evidence of any granulation tissue (fig. 3 *A*). In the alveolar walls, however, a larger number of reticuloendothelial cells were seen together with a few polymorphonuclears. Again a few eosinophils were found. The capillaries were distended with red blood cells. The blue-staining material became less toward the periphery of the focus, and finally in the surrounding alveoli nothing but a more or less homogeneous, slightly acidophilic substance was seen, probably precipitated albuminous fluid such as is not infrequently seen in edema. As has been stated, the amount of necrosis of the alveolar walls was actually small. There was no true tubercle formation, no development of epithelioid cells and, in particular, no giant cells. Even lymphocytes were absent. The lung tissue between the lesions showed thickening of the alveolar walls, this thickening being due to infiltration with the type of cell described, but the alveoli themselves were open and contained air. It was striking that the bronchi and even the smaller bronchioles, blood vessels and lymphatics were not involved. At the hilus the main bronchi were free.

Acid-fast stains revealed that the blue-staining material was composed of myriads of tubercle bacilli (fig. 3 *B*). The masses were so large that they could be identified under the low power dry lens (× 200). The organisms were well stained, were in sheaves, did not show granulation but were only in part gram positive. Alveoli surrounding the foci also contained the organisms, although in smaller numbers. However, the alveolar walls, bronchi and other structures were free.

The Weigert stain for fibrin did not show fibrin in the alveoli. The tubercle bacilli stained positively with this method. In controls, however, it was possible to distinguish between them and true fibrin.

The lymph nodes, although in the gross their structure was not altered, were considerably distorted microscopically, in part destroyed, by collections of cells resembling those seen in the lung. Here, too, eosinophils were among the few polymorphonuclears present. Necrosis was somewhat more advanced. The change here, too, occurred in foci, frequently near the peripheral sinus rather than in the depths of the node. In the center of these foci the cells stained poorly and there was considerable chromatin dust. However, the process had not advanced beyond the lymph node. The nodes involved were the left bifurcation, the right paratracheal, the right bifurcation and the bronchopulmonary nodes. The lymph nodes in the posterior mediastinum along the course of the thoracic duct were small but also involved. In the superior gastric lymph nodes there was hyperplasia but no evidence of the type of disease described.

The umbilical arteries and veins showed no thrombosis in the section studied. There was, however, an obliterating process with calcification which almost completely closed the lumen of the artery. The ductus venosus Arantii showed a thrombus containing many cells of the reticulum type. No evidence of organization

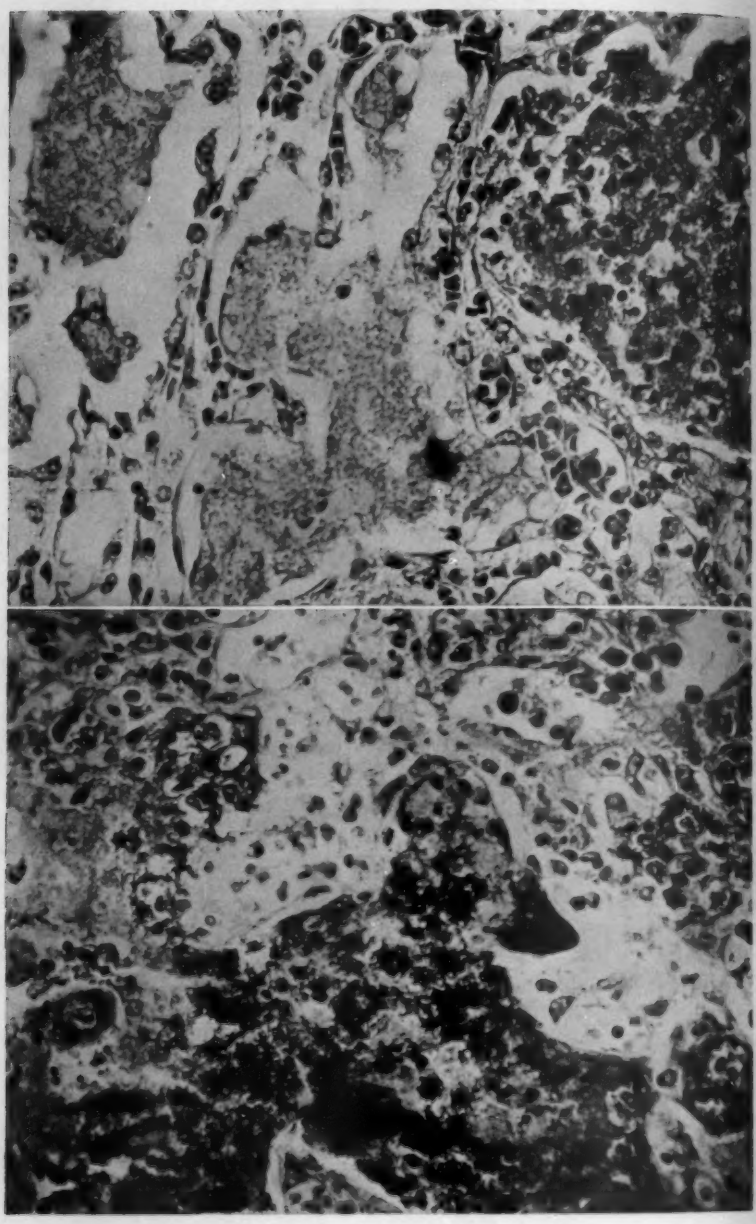

Fig. 3.—Upper: typical example of alveolitis; hematoxylin and eosin; × 445. Lower: Ziehl-Neelsen stain of lung; × 445.

could be seen. There was no adhesion to the wall and no necrosis. In the mass of the clot a few polymorphonuclears were seen and some acidophilic fibrillar material. Acid-fast stains showed no organisms. This mass was found in only one section along the ductus; the rest showed no pathologic change.

Sections of the gastrointestinal tract, heart, thyroid, hypophysis, thymus, pancreas, adrenal, kidney and spleen showed no disease. Acid-fast stains of kidney and spleen were negative.

Careful search failed to reveal any focal necroses in the liver. There were, however, considerable granularity and vacuolation of the cytoplasm of the liver cells. Acid-fast stains revealed no organisms.

Bone marrow from a vertebra disclosed no focal necrosis. The marrow was hyperplastic. Perhaps the most striking thing was the tremendous number of eosinophils and eosinophilic myelocytes.

The final diagnosis was congenital primary pulmonary tuberculosis (aspiration type). This was supported by the following evidence: The autopsy of the infant demonstrated no tuberculosis of any other organ except the lung and its lymph nodes. The process consisted essentially of alveolitis in acinonodose form with an exudate of few polymorphonuclear leukocytes and a preponderant number of mononuclears of the reticuloendothelial type. There was a perifocal edema with no cellular exudate. Tremendous numbers of tubercle bacilli were found in and about these lesions. The foci were distributed equally throughout both lungs and were larger and more frequent toward the base. The alveolar walls, perivascular and peribronchial tissues and bronchi were not affected; the process was definitely and exclusively in the alveoli. This excludes the possibility of a hematogenous distribution by way of the ductus venosus Arantii. Although it is well known that in the course of miliary tuberculosis tubercle bacilli may be excreted into the alveoli and then produce tuberculous bronchopneumonia of miliary form and distribution, yet typical interstitial tubercles were also found in large numbers in all these cases. The fact that the lesions were larger and more frequent toward the base is not in agreement with what the pathologist finds in miliary tuberculosis. Lastly, the absence of tubercles in other organs and the fact that the liver and the portal nodes were not affected exclude distribution by way of the umbilical vein and liver. The intestine and the mediastinal nodes were unaffected; ingestion is therefore out of the question. It appears that the only mechanism by which the infant could have been infected is that of aspiration. Postpartum infection cannot adequately explain the process. Not only is there no clinical evidence of contact; in addition, it is in the highest degree improbable that a postpartum tuberculous infection could lead to death within the period of four weeks. For this, if for no other reason, the breast milk could not have been the source. The extensive seeding of the lungs with enormous numbers of bacteria necessarily required to produce the picture described cannot

have been the result of any ordinary transient contact. The proper conditions for such infection are found in contamination of the liquor amnii. The histologic examination of the curettings from the mother demonstrated tuberculous endometritis with well developed, in part caseating tubercles, which might have been present at or before the birth of the child.

The cellular response to the infection was that characteristic of the early stage of primary infection. In fact, the individual lesion which we have described was exactly like that described by Zarfl [17] in a case of postpartum aerogenous infection in a very early stage, in which the child died of an intercurrent disease at the age of 24 days. The minute primary focus was examined at a stage when gross evidence of lymph node involvement had not yet appeared. The absence of tubercle formation in these early stages, which has also been described for animal experiments, substantiates the opinions of those who maintain that the tubercle is not the primary reaction of the body to the tubercle bacillus but rather a reaction acquired in the course of the disease. As in Zarfl's case of an early primary focus and as in several of the cases of congenital aspiration tuberculosis,[18] the lymph nodes were small. We therefore agree with Siegel [9] that the process is a widespread focal primary infection of the lungs.

The case reported is strikingly similar to the ones described in the literature. In most cases the disease of the mother was far advanced at the time of birth; usually hematogenous distribution could be demonstrated either clinically or at autopsy. However, in the case reported by Giuliani [3] death occurred two years after the birth of the tuberculous child. Although the placenta was not satisfactorily examined in any case, tuberculosis of the internal genitalia was demonstrated in 4 cases. The child was usually premature. Commonly no signs or symptoms of a severe illness were noted until shortly before death, when cyanosis, dyspnea, asphyxia, cough and a rapid decline set in. This extraordinary tolerance of the body toward the constitutional effects of tuberculous infection is well known to pediatricians. Death usually occurred between the sixteenth and the twentieth day, although in Zarfl's [11] case the child lived to the forty-third day.

For the most part the authors who have published cases of congenital tuberculosis of the aspiration type have stated that the infection occurred at the time of birth. This assumption rests on the widely accepted belief that respiratory movements do not begin in the fetus before parturition sets in. This, however, is not so. Aspiration of infected liquor amnii may take place at an earlier period. In the report of Andrewes' [6]

17. Zarfl, M.: Ztschr. f. Kinderh. **5**:303, 1913.
18. Giuliani.[3] Siegel.[9]

case it is stated that the child died "shortly after birth." In Giuliani's [3] case the infant showed dyspnea and asphyxia from the time of birth. The mother in the case observed by Couvelaire and others [10] was delivered by cesarean section, and thus the child was not subject to the periods of asphyxia in utero which some authors regard as important in determining the aspiration of liquor amnii. These cases demonstrate that the infection may well occur before parturition. In any individual case, however, it may be difficult to state the exact time of infection. We agree with Kraus,[8] who stated that "all factors which affect the child or parts of its body after it has passed the introitus are according to common usage to be regarded as acquired, but those acting before can be regarded as congenital."

SUMMARY

A case of congenital pulmonary tuberculosis of the aspiration type is described. Reports of 7 unequivocal cases of the same type were found recorded in the literature.

SUBDURAL OR INTRADURAL HEMORRHAGES?

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Recently claims have been put forward that what pathologists have been calling subdural hematomas are actually intradural hematomas. Before discussing this question it is necessary to consider what one means by the term "subdural hematoma." Minor hemorrhages with relation to the dura or the subdural space have no clinical significance, do not constitute hematomas and can be excluded.

Hemorrhages into the subdural space may arise from many causes. The commonest type is met with in the fractured skull, in which the source of the hemorrhage is almost exclusively a laceration of the arachnoid and brain. Rarely a laceration of the dura may be a source in this group. Hemorrhages in the newborn are usually due to injuries sustained at birth and commonly arise from the falx or from the tentorium. The cause of the clinical picture is not the hemorrhage into the membranes but the mass of blood which escapes through rupture of the membranes and accumulates in the subdural space. Occasionally tumors may be responsible for extensive subdural hemorrhages. Essential cerebral hemorrhages have been known to rupture into the subdural space. In addition, syphilitic or tuberculous processes may produce true hemorrhagic pachymeningitis.

Quite distinct from the group of cases just described is a series of cases in which massive hemorrhage into the subdural space occurs, usually following traumatism, which is in most cases minor and which is unassociated with fracture of the skull or laceration of the brain. The traumatism may not be to the head, and may be so minor that it is overlooked. These hemorrhages occur usually in adults. Alcohol is a factor in many cases, and the hemorrhages have an apparent relation to C avitaminosis. It is with this type of hemorrhage that the present paper deals.

The regional localization of free blood in the subdural space is quite constant, and is significant in the evolution of hematoma. Whatever its source, the mass of the blood will, with rare exceptions, be found over the convexity, where it may attain a thickness of 1.5 to 3 cm. The usual source of the blood is above the tentorium, and the downward pressure on this membrane will prevent the escape of blood into the cerebellar fossa. In the standard case there will be found a thick layer of blood over

From the office of the Medical Examiner of Suffolk County, Southern District

the convexity and a thin layer over the base in the anterior and middle fossae and less constantly over the upper surface of the tentorium and, to a lesser degree, over the ipsilateral surface of the falx. Just as the tentorium seals off the cerebellar fossa, so the tentorium and the falx will frequently prevent escape of blood to the subdural space of the opposite side. In about 15 per cent of the cases this mechanism fails, and the process is bilateral.

The Massachusetts medicolegal system gives exclusive opportunity to medical examiners to investigate the causes of deaths supposed to be due to violence. Because of the frequency of subdural hemorrhages, which in some years made up 10 per cent of intracranial hemorrhages in my service, I reported in 1934 a series of 50 cases.[1] This series covered the gamut of changes which arise following escape of blood into the subdural space and made it possible to follow the evolution of the process in its various stages from the beginning to the end result, i. e., from fluid blood to complete organization. A series of cases seen since confirms the conclusions arrived at from study of the first series.

Because of the peculiar character of the attempt to organize a subdural hemorrhage (with activities in this respect limited to the relatively avascular dura, while the highly vascularized arachnoid remains inactive), a study was made of the subdural space and its linings.[2] This study disclosed that the subdural space is not a serous space, as was formerly believed. On one side is the highly differentiated pia-arachnoid, a complex membrane and an essential part of the central nervous system, which it serves to protect both physically and chemically. On the other side is a simple fibroblastic membrane —the dura—which is the inner periosteum of the skull and which serves as a support to the brain, and encloses the venous sinuses. It is a part of the skull complex just as the pia-arachnoid is a part of the brain complex. The simplest explanation of the subdural space is that the skull with its lining dura enters into articulation with the brain and its covering pia-arachnoid. From this point of view the dura is the lining of an articulation and is therefore a simple fibroblastic membrane.

The character of the lesions dependent on subdural hemorrhages divides them into five broad groups, each marking a stage in the aging and progress of the hematoma. The first stage is found in cases in which death is prompt following the hemorrhage; in this stage the blood in the subdural space is fluid (fig. 1 *A*). The process is usually unilateral, molding and compressing one hemisphere and flattening the surface of the other hemisphere against the skull. In the second stage the blood has clotted but is not adherent to the dura (fig. 1 *B*). In the

1. Leary, T.: J. A. M. A. **103**:897, 1934.
2. Leary, T., and Edwards, E. A.: Arch. Neurol. & Psychiat. **29**:691, 1933.

third stage organization of the clot has begun from the dural side. The clot, which is now discolored copper brown and softened, is adherent to the dura by the formation of a single outer neomembrane; i. e., it is still uncovered on its inner surface (fig. 1 *C*). The fourth stage is marked by a double neomembrane enclosing the clot (fig. 1 *D*). The fifth stage shows healing, more or less complete, by fusion of the outer and inner neomembranes, and is of no significance clinically. The hemorrhage is usually unilateral but may be bilateral. Rarely there may be manifest differences in age between the lesions on the two sides.

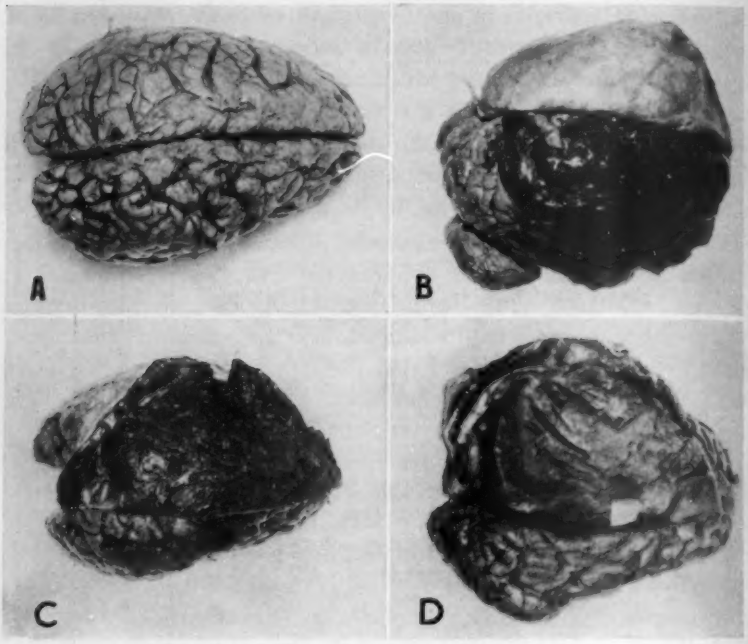

Fig. 1.—*A*, subdural hemorrhage, first stage—fluid blood. Note molding of the right hemisphere, with compression and flattening of the surface of the left hemisphere. *B*, second stage—early currant jelly clot; no adhesion to dura. *C*, third stage—softened clot discolored brown and adherent to the dura by an external neomembrane. Note the rough inner surface of the naked clot. *D*, fourth stage, clot wholly enclosed within a double neomembrane. A window has been cut through the thinner portion of clot and the smooth opaque white dura exposed. Note the compression deformity of the brain.

In contrast to the old belief that the subdural hematoma always runs a chronic course, pathologists now know, to quote Munro,[3] that: "The so-called 'chronic subdural hematoma' was actually only a late

3. Munro, D.: Cranio-Cerebral Injuries: Their Diagnosis and Treatment, New York, Oxford University Press, 1938, p. 128.

stage of the acute injury, in a patient that had been fortunate enough to survive in spite of a complete lack of treatment."

Death in the first stage (fluid blood) occurs usually within a few hours of the vascular rupture. In the second stage (free, unadherent clot) death, if it occurs, follows usually within one to five days. The third stage (single external neomembrane, i. e., clot adherent on its outer surface to dura) is found one to three weeks after the vascular rupture. The fourth stage (clot enveloped in double neomembrane) is found three weeks to years after the original hemorrhage. The fifth stage (fused neomembranes) is a more or less completed repair process and is a casual finding at autopsy, probably years after the original hemorrhage. Death during the first two stages is usually due directly to the subdural hemorrhage. In the third stage death may be due directly to the hemorrhage, or it may be due to associated conditions, such as alcoholism or infection. The stage most commonly met with is the fourth, with the hematoma enclosed in completed membranes. It is this stage which is most important clinically. Death at this stage is frequently due to secondary hemorrhages, which may rapidly increase the intracranial pressure. The secondary hemorrhages may be limited wholly within the neomembranes or may rupture through the thin inner neomembrane into the subdural space. The breaking down of the complex blood proteins into simpler molecules may greatly increase the osmotic pressure within the double neomembrane, as Munro [4] has shown, and may produce a cystlike sac in which the largely fluid contents are under great tension, often causing an exacerbation of symptoms.

Intermediate steps in the evolution of the process are found. For example, the extension of the neomembrane over the naked internal surface of the clot may be incomplete in the third stage, the smooth surface of that portion of the clot which is covered by the growing membrane contrasting with the rough surface of the still uncovered portions of the clot (fig. 2). Apart from changes in color and consistency, marked alterations, including partial or more or less complete liquefaction of the clot, occur mainly after the clot has been enveloped in a double neomembrane.

The blood in subdural hemorrhages is distributed over the dural surface above the tentorium. The layer of blood over the base and tentorium is thin as compared with that over the convexity. Nothing which can be stigmatized as a hematoma ordinarily appears in these locations. The presence of the blood, however, stimulates repair activities in these sites as well as over the convexity. Because of the thinness of the blood layer, repair is more rapid and successful than

4. Munro, D.: New England J. Med. **210**:1145, 1934.

over the convexity. However, even after repair is complete, coloring of the dura in these locations by blood pigment may persist for long periods.

The relatively avascular dura can produce only a comparatively feeble growth of granulation tissue. Particularly is there difficulty in venous drainage from the new tissue. The passive hyperemia due to this imperfect venous drainage produces wide dilatation of the capillaries of the granulation tissue, which frequently show a diameter of over 100 microns. Putnam and Cushing [5] titled these "giant capillaries." In the later stages of repair, when these vessels are outlined by masses

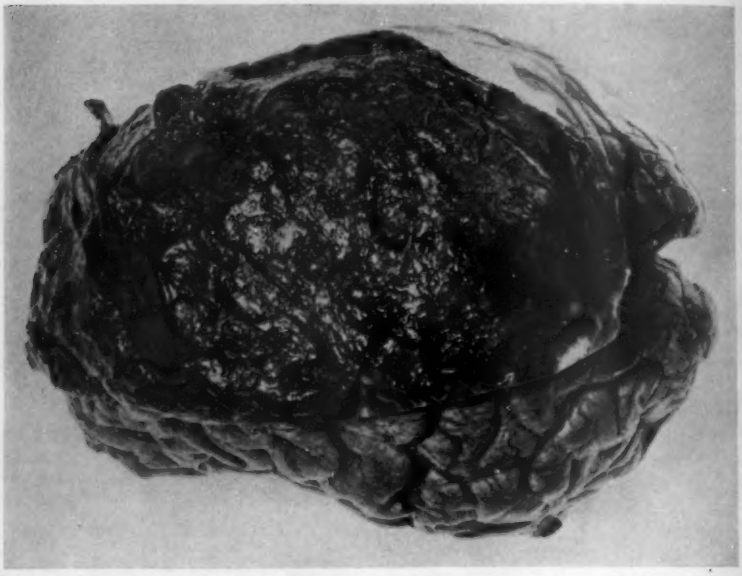

Fig. 2.—Third stage—intermediate. A growth from the external neomembrane is extending over the inner surface of the clot, particularly on the right. Note the relatively smooth area on the right and the rough uncovered clot. Smaller smooth regions in figure 1 *C* mark the advance of the proliferating tissue.

of macrophages filled with blood pigment, they take on a sinusoidal character. These capillaries, poorly supported, are the source of secondary hemorrhages. I have been able to demonstrate actual points of rupture of these vessels in the neomembrane.

In a word, the persistence which is the outstanding characteristic of subdural hemorrhages as compared with hemorrhages elsewhere in the body arises from the fact that the burden of organizing and removing the clot is thrown on a poorly vascularized dense connective tissue

5. Putnam, T. J., and Cushing, H.: Arch. Surg. **2**:329, 1925.

membrane that is in contact with only one surface of the clot and has difficulty in carrying out the assignment. In serous spaces organization of clot proceeds from all surrounding surfaces. Here the effort at repair is from one surface alone.

SUBDURAL OR INTRADURAL?

To paraphrase Klotz [6] discussing the etiology of arteriosclerosis: "The mode of origin or the previous course of" *a closed subdural hematoma (fourth stage)* "cannot be determined by a study of the old lesions alone, and it is futile to hold controversy over such an indeterminable problem. A study of the earliest stages of the lesions in the tissues of man is still the most secure upon which to base conclusions."

There have recently appeared three papers in which it is claimed that so-called subdural hematomas are actually intradural. Hannah [7] cited 3 cases: a case in which there were petechial hemorrhages into the dura at the site of an operation, and 2 cases in which a double neomembrane was present. He injected oxalated blood into the dura in an attempt to reproduce the lesions as intradural lesions, but the largest bleb containing blood that he could make was 0.5 cm. in diameter. Moreover, section of such a bleb disclosed torn strands of fibrous tissue crossing the bleb from its inner to its outer surfaces.

Kaump and Love,[8] following Hannah's suggestion, studied 30 cases of so-called subdural hematoma. The lesions in the traumatic group varied from an extensive hematoma which had compressed the brain to only a small area of hemorrhage. In these cases the hematoma was enclosed within a membrane. In the group of spontaneous hematomas there was variation in size from massive bilateral tumors, each containing 300 cc. of material and compressing the brain, to small areas of hemorrhage scattered through the dura. Of 3 patients with spontaneous hematomas who had given clinical and serologic evidence of syphilis, the lesions in 1 patient were small and more or less discrete, and those in 2 were large. In 3 patients with associated blood dyscrasia bilateral hematomas were present—in 1 patient large, in the other 2 consisting of conglomerate patches of intradural hemorrhage.

It is evident that in this series are included minor hemorrhages, probably intradural, together with massive hemorrhages originally subdural, which had become enveloped in a double neomembrane.

Baker [9] in a general review of subdural hematoma recorded 31 cases, without detailed descriptions, but he evidently included obstetric

6. Klotz, O.: J. M. Research **31**:409, 1915.
7. Hannah, J. J.: J. Nerv. & Ment. Dis. **84**:169, 1936.
8. Kaump, D. H., and Love, J. G.: Surg., Gynec. & Obst. **67**:87, 1938.
9. Baker, A. B.: Arch. Path. **26**:535, 1938.

traumatic hemorrhage of the newborn. He referred to cases of pernicious anemia in which petechial hemorrhages were found in other organs, and apparently included the whole series of hemorrhagic lesions which may appear in or about the dura.

The group of subdural hemorrhages which has clinical importance is made up of relatively massive hemorrhages. Failure to differentiate these massive hemorrhages from the minor petechial or conglomerate petechial hemorrhages into the dura serves but to complicate and confuse the issue.

Physical Objections to the Intradural Theory.—A practical physical objection to the conception of the hematoma as intradural is the difficulty of splitting the dura so that it could contain several hundred cubic centimeters of blood within its meshes or so that this mass of blood could be thickest over most of the convexity on one side in the cases in which it is unilateral and over a similar area on both sides in cases in which it is bilateral. Moreover, there is usually a much thinner layer of blood over the base and tentorium as well, which must be considered. The practical impossibility of so shearing the dura into a thicker outer layer and a delicate translucent inner layer over such extensive regions is manifest. The splitting must occur with such perfection that there is no escape of blood into the subdural space.

Hannah has illustrated the difficulty of forcing blood between the meshes of the dura. Practical tests by me, using a syringe with a force greater than any conceivable blood pressure, have demonstrated the impossibility of producing the widespread splitting of the dura necessary to include large masses of blood within its meshes. It is true that petechial hemorrhages may occur in the dura. It is also true that these hemorrhages may occur in agminated masses which appear to be continuous, but section will disclose that the thin zone of hemorrhage is broken up by bands of dural tissue into a series of units, as Hannah found. In contrast to this, in the fourth stage of the subdural hematoma the sac enclosing the blood is monolocular, without partitions, except when a secondary hemorrhage occasionally results in a bilocular sac.

Because of the poor quality of the granulation tissue produced from the dura, the attachments of the outer neomembrane to the dura tend to be less than firm, frequently showing only delicate strands of fibrous tissue securing the outer neomembrane to the dense inner layer of the dura. It is this fact that makes the removal of a subdural hematoma a feasible operative procedure. If the hemorrhage were intradural, it would be wholly impracticable to attempt to remove the extensive mass by operation through a limited opening in the skull.

The progressive character of the stages in the evolution of the subdural hematoma discredit a belief that the massive hematoma is

intradural. In the first and second stages (free blood-free clot) there can be no doubt that the hemorrhagic materials lie in the subdural space. In the third stage the clot is adherent to the dura by organization (from the dura) of its outer surface, but the inner surface remains naked clot. In the intermediate third stage the growth from the outer (dural) neomembrane is seen spreading over the inner naked surface of the clot to enclose it. (See fig. 2.) In this stage also there cannot be reasonable doubt that the hemorrhage was subdural. It is only in the fourth stage, after complete envelopment of the clot, that any confusion could arise as to whether the hemorrhage was intradural or subdural. To reason backward from this advanced stage to the causation of the lesion without study of the early stages is hardly justifiable.

SOURCE OF THE HEMORRHAGE

It is apparent that the source of the subdural hemorrhage can be determined only in cases in which organization has not as yet covered up whatever evidence of the source may have been present. It is only in the first and second stages, therefore, that such evidence may be found.

Although the blood lies in the subdural space, as shown in foregoing sections of this paper, it is possible that the source of the hemorrhage could be ruptured dural vessels. The dura is a dense membrane with a smooth, pale inner surface. Hemorrhage into its tissues about the point of rupture of a vessel should be easily recognized, as are hemorrhages that have occurred in the newborn as a result of injuries at birth. In such cases the hemorrhage is interstitial, and space is made for the blood by tearing apart the dural connective tissue. In a recent case of fatal hemorrhage resulting from an injury at birth, in which the infant died fifty-two hours after birth, the tentorium, in the meshes of which the fatal hemorrhage had originated, showed a maximum thicknesss of 0.4 cm. Rupture through the surface layers of the tentorium had resulted in the accumulation of 80 cc. of clot free in the subdural space. This clot was the cause of death. The hemorrhage into the tentorium alone was not great enough to influence markedly intracranial pressure. Microscopically, the density of the fibrous tissue in the tentorium and the necessity of tearing apart the collagenous fibers before blood can collect make it evident that intradural hemorrhage must be of necessity limited.

In my cases of hemorrhage of the adult brain in the first and second stages careful inspection of the dura after removal of the blood or the clot disclosed no dural source of the hemorrhage. On the other hand, I have been able to demonstrate the source in bridging veins or at the point of junction of these veins with arachnoid veins in 12 cases.

BRIDGING VEINS

Hannah limits the term "bridging veins" to the portions of the superior cerebral veins which cross the subdural space to the longitudinal or the sagittal sinus. The term as used in this paper is applied to veins which cross the subdural space away from and do not join directly any sinus. Browning [10] described the veins which spring from the arachnoid to the dura over the frontal convexity 1 to 4 cm. from the sagittal sinus. Mittenzweig [11] found a vein ruptured in its course 4 cm. from the sagittal sinus over the right frontal lobe in a case of subdural hemorrhage with fluid blood. He made a study of 200 heads and in 59 found aberrant veins which crossed the subdural space far from the sagittal sinus over the frontal convexity, and in 9, veins which crossed over the posterior convexity.

Apart from these studies, which refer only to veins over the convexity of the brain, the literature contains little on the subject. The standard textbooks on anatomy do not mention these veins. The veins in question are so delicate and so easily broken that in removal of the brain they are ordinarily overlooked. The frequency with which they appeared to be the source of subdural hemorrhage led me to investigate them.

These veins vary markedly in size and in thickness of wall. When put on tension, they are threadlike structures, for the most part less than 0.1 cm. in diameter. When relaxed and filled with blood, some may measure up to 0.2 cm. in diameter. They occur singly or in groups and are not usually united at their dural ends as the superior and inferior cerebral veins frequently are. They occur most commonly in four locations:

1. Crossing from the arachnoid of the convexity, usually in the frontal region, most commonly 1 to 4 cm. from the sagittal sinus, as Browning has described. They vary in length up to 1 cm. or more and may occur in considerable numbers. They are present in smaller numbers and less commonly over the parietal convexity, where they tend to be longer than those found over the frontal convexity. They are rarely found over the standard field for decompression operations and the whole lower convexity but are met with occasionally in operations over the convexity.[12]

2. Traversing the space from the basilar arachnoid of the temporal lobe to the tentorium and the dura over the middle fossa. The inferior cerebral veins empty largely into the transverse sinuses. They occur

10. Browning, W.: The Veins of the Brain and Its Envelopes: Their Anatomy and Bearing on the Intracranial Circulation, Brooklyn, F. B. O'Connor, 1884.

11. Mittenzweig: Neurol. Centralbl. **8**:193, 1889.

12. Putnam, T.: Personal communication to the author.

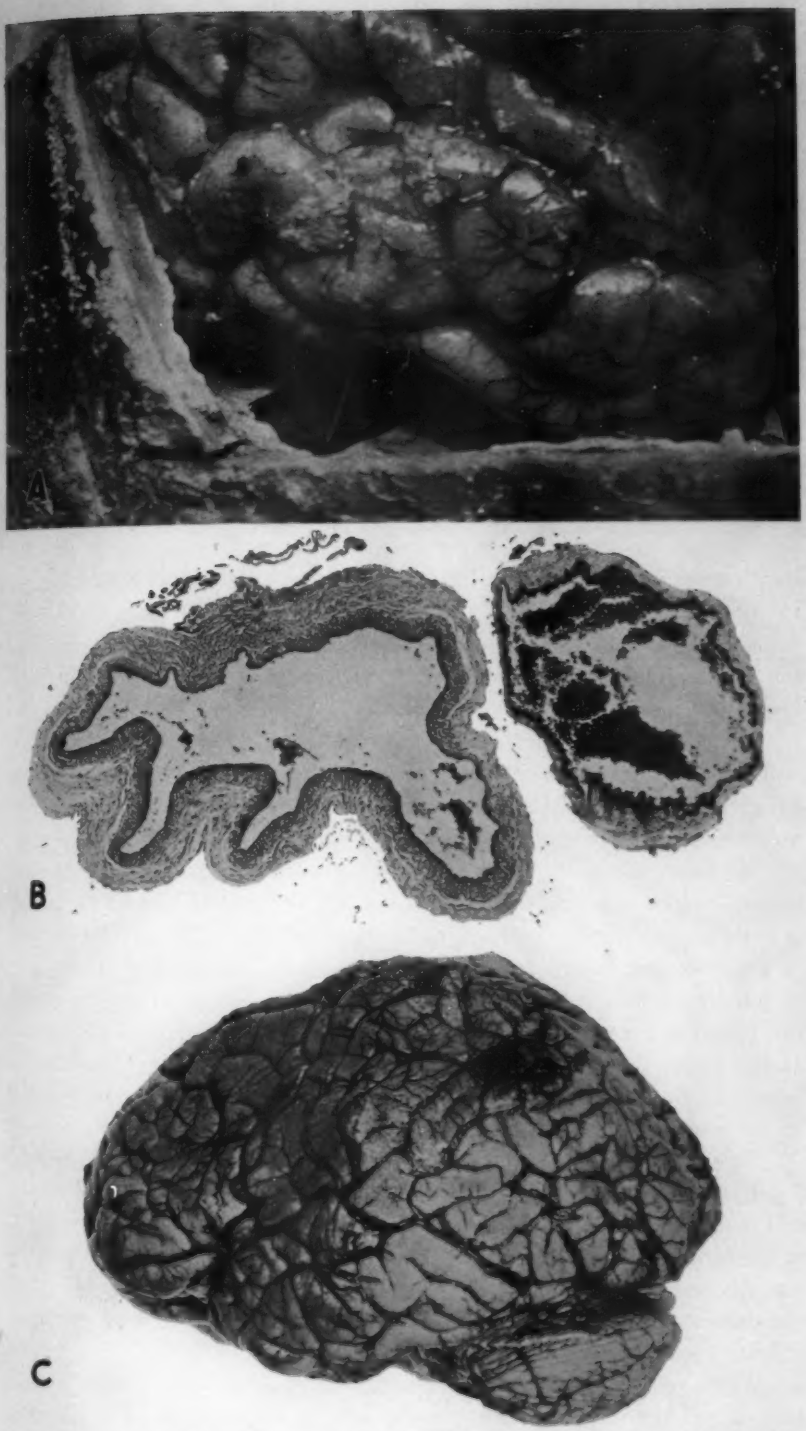

Fig. 3.—*A*, a bridging vein over the base of the temporal lobe. A piece of blue paper has been placed over the dura of the base for contrast. *B*, photomicrograph of bridging veins (see text). *C*, brain showing tell-tale hemorrhage into the arachnoid about the source of hemorrhage, i. e., a bridging vein which had broken at its junction with an arachnoid vein in the posterior parietal region. Note the deformation of the brain.

as groups of veins, in largest numbers along the outer border of the posterior temporal and the occipital lobe. Aberrant veins are almost constant, occurring away from the location of the sinuses, over the midbase of the temporal lobes. They are frequently single vessels—sometimes they occur in pairs (fig. 3 *A*). In some cases it is possible to follow a vessel in the upper layers of the dura for 2 cm. before it empties into the superior petrosal sinus.

3. From the frontal pole of the temporal lobe to the dura over the sphenoid bone. The middle cerebral veins empty into the cavernous sinus. The frontal pole of the temporal lobe varies remarkably in its relation to the dura. Not only venous but fibrous attachments may anchor the pole firmly to the sphenoidal dura. On the other hand, the connections may be delicate and wholly venous. The veins may be multiple or single, large or small. Aberrant veins occur in this location but are of little significance in relation to subdural hemorrhages, in my experience.

4. Subtentorial veins are often aberrant but are not important from the standpoint of subdural hemorrhages, which arise almost exclusively above the tentorium.

In general, when aberrant veins are found in one of these loci they will also be found in the other regions described. They are most constant over the basilar surface of the temporal lobe. In some skulls the number of these veins may be great, while in others there are few.

Microscopically, the walls of bridging veins are made up of fibrous tissue, a more condensed layer surrounding the lumen in a band, which is sometimes definite, with an outer layer of looser connective tissue. A single elastic lamina lies next to the vascular endothelial lining and is sometimes doubled. Occasionally, small fragments of elastic tissue are found in the inner more compact connective tissue layer, and rarely, in the looser outer tissue. As in all of the cerebral veins, there is no unstriped muscle tissue, i. e., no media. The outer surface is covered by an endothelium-like layer.

Figure 3 *B* shows two discrete bridging veins which were mounted together with others and cut in cross section. The larger vessel to the left is thick walled, almost empty and contracted. The vessel to the right is moderately distended with blood and illustrates the irregularity in the thickness of the wall frequently found in injected vessels. The slenderness of the barrier between the contained blood and the free subdural space is evident. The margin of safety is small.

When put on stretch, the veins may be drawn out into long elastic threads. They tend to rupture at the arachnoid junction, in which case small openings are torn in the arachnoid veins with which they are connected. This is significant, since small tell-tale hemorrhages into the

subarachnoid space tend to arise about the site of rupture during life and mark the source of subdural hemorrhages (fig. 3 *C*). If rupture occurs away from the arachnoid junction, it is often difficult or impossible to determine the source of the hemorrhage.

As one studies these delicate veins the wonder grows, not that they rupture, but that they do not rupture more commonly. In connection with C avitaminosis, they are perhaps the veins which rupture most frequently in prescurvy conditions.

ARACHNOID DRAINAGE IN FRACTURED SKULLS

An interesting observation in a field closely related to this one is that subdural hemorrhages which arise from laceration of the arachnoid and the brain (associated with fracture of the skull) rarely persist for long periods, in contrast to the subdural hemorrhages I have described. If the victim survives the injury by as much as a month and then dies (from bronchopneumonia or infection usually), examination will reveal *plaques jaunes* and adhesions of the arachnoid to the dura over the site of brain injury but no subdural blood or clot or dural neomembrane. The explanation of the disappearance of the blood appears to be that the efficient arachnoid drainage system has been opened up by the laceration and the subdural space cleared. Rare exceptions to this relatively rapid removal of blood under these conditions are found in senile arteriosclerotic persons, in whom all functions are less efficient.

This observation makes it evident that for the production of a persistent subdural hematoma it is necessary that the arachnoid be essentially intact. The minute openings into arachnoid veins at the point of rupture of bridging veins are not significant in this respect.

A pertinent suggestion from this observation is that an opening into the arachnoid drainage system might be desirable in operations on subdural hematoma in which removal of all of the hematoma is not operatively possible. Like many suggestions, this has its limitations. Opening of the arachnoid will result, under present day surgical possibilities, in adhesions to the dura at the location of the arachnoid opening, and adhesions are a potent cause of epileptic seizures. Some day, perhaps, an acceptable tissue membrane will be found that can be placed between the dura and the arachnoid to prevent adhesions.

SUMMARY

Evidence is presented to prove that massive subdural hemorrhages are actually subdural and not intradural. The evolution of the process from free fluid blood to completely enveloped hematoma is illustrated.

The common source of subdural hemorrhages is ruptured bridging veins.

When in fracture of the skull there is laceration of the brain, the blood which accumulates in the subdural space is removed with relative rapidity through the efficient arachnoid drainage system. As a result, chronic subdural hematoma does not arise under these conditions. An unbroken arachnoid appears to be a necessary element in the production of subdural hematoma.

ORIGIN AND SIGNIFICANCE OF BINUCLEATE PURKINJE CELLS IN MAN

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There have been a number of reports on the division or apparent division of the nucleus of the nerve cell not only in the sympathetic but also in the central nervous system. Binucleate cells have been found in a variety of pathologic conditions, especially in psychoses, such as schizophrenia, and in progressive paralysis. The number of reports on binucleate Purkinje cells in man is, however, small. Schröder [1] in 1911 reported 2 cases of schizophrenia in which binucleate Purkinje cells were present. Von Sántha [2] in 1930 reported the presence of binucleate Purkinje cells in 2 of 13 cases of schizophrenia which he examined. Rubinstein reported binucleate cells in the cerebellum in some of the 35 brains from psychotic patients which he studied.

Binucleate Purkinje cells have been discovered as a senile phenomenon in the rat by Inukai [3] and by Loo [4] and in the mouse by Andrew.[5] These authors agree that the binucleate condition of the Purkinje cells in rodents is brought about by an amitotic division of the nucleus. Binucleate cells are common in the autonomic system of rodents. There is evidence that the amitotic division of nuclei of some of the Purkinje cells of rats and mice may begin at rather an early age, although the great increase in binucleate cells occurs only in advanced age. Inukai found Purkinje cells with elongated nuclei with two nucleoli in albino rats aged 200 days and double or even triple nuclei in a rat aged 730 days, 1 aged 1,017 days, and 1 aged 1,085 days.

In a study of 100 Purkinje cells each from 19 mice of various ages, Andrew [5] found a total of 22 binucleate cells in the brains of the 4 senile specimens in the group and none in the brains of the 15 other specimens, none of which was over 290 days of age. In a more recent study by Andrew [6] binucleate cells were found in 2 mice of 324 days of age but only about one-quarter the number of those in senile animals

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1. Schröder, cited by Rubinstein, B. G.: Acta med. Scandinav. **81**:215, 1934.
2. von Sántha, K.: Ueber die Entwicklungsstörungen der Purkinjeneurone, Arch. f. Psychiat. **91**:373, 1930.
3. Inukai, T.: J. Comp. Neurol. **45**:1, 1928.
4. Loo, Y. T.: J. Comp. Neurol. **67**:423, 1937.
5. Andrew, W.: Ztschr. f. Zellforsch. u. mikr. Anat. **27**:534, 1937.
6. Andrew, W.: Am. J. Anat. **64**:351, 1939.

(691 days of age and over) studied at the same time. One definitely binucleate cell was found in a mouse 160 days of age. The binucleate condition of the Purkinje cells in mice and rats seems to be, then, a condition brought about by amitotic division of the nucleus, probably always preceded by division of the nucleolus, and occurring in full grown and most commonly in senile animals.

In a study of the Purkinje cells in man from birth to senility I[7] described a number of changes in senile persons which resembled rather closely the changes in senile mice and rats. I failed, however, to find the binucleate condition in any of a large number of Purkinje cells in the cerebellums of 34 persons. Of these persons, 5 were of advanced age: 69 years, 70 years, 72 years (2) and 80 years, respectively.

MATERIALS AND METHODS

Since the publication of the earlier work on the human Purkinje cell[7] specimens of cerebellum from 6 additional persons have been prepared and examined. These

Summary of Data on Subjects Studied

Age	Sex	Subject	Cause of Death
5 months	♂	Negro	Neurocytoma of abdominal ganglion
13 months	♂	Negro	Meningitis — influenzal meningitis
22 years	♀	Negro	Cerebrospinal syphilis, terminal pneumonia
27 years	♂	White person	Abscess of brain
49 years	♀	Negro	Cirrhosis of liver
56 years	♂	White person	Fracture of skull and cerebral hemorrhage

specimens, being autopsy material, were necessarily fixed at different times. The fixative employed was solution of formaldehyde U. S. P. diluted 1:10. The specimens were kept in the solution for periods ranging from one week to two months. They were then carried through the processes of dehydration, clearing, infiltration and embedding in paraffin together, sectioned, and stained in the same glass slide tray.

The specimens, the age and sex of the patient and the cause of death are listed in the table.

The stain employed for Nissl material was cresyl violet. In addition, pieces from each specimen were prepared by the Da Fano method of silver impregnation in the manner described by me in a previous article,[6] which had been found to give a uniformly good picture of the dendrites of the Purkinje cells.

OBSERVATIONS

The interesting finding among the specimens from this group was the occurrence of a very considerable number of binucleate Purkinje cells in the cerebellum of the 22 year old Negress who died of cerebrospinal syphilis.

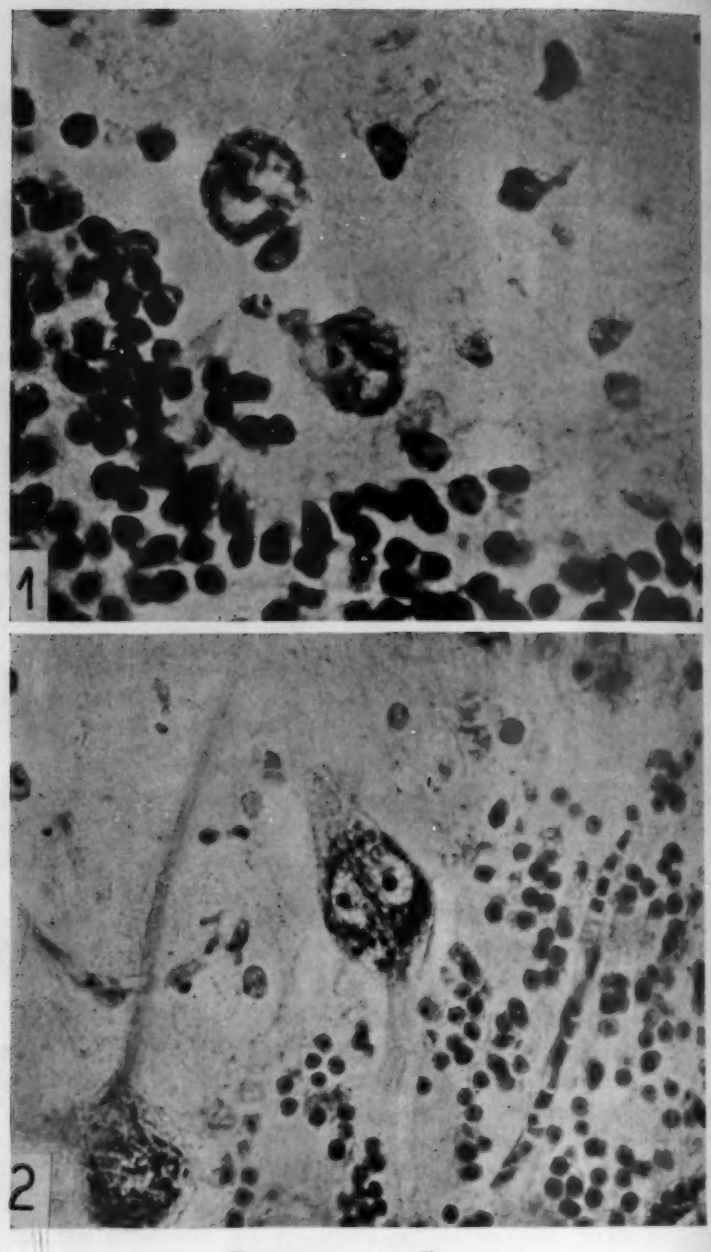
7. Andrew, W.: Ztschr. f. Zellforsch. u. mikr. Anat. **28**:292, 1938.

This patient's condition had been diagnosed as dementia paralytica with meningeal involvement and pelvic inflammatory disease. At autopsy the meninges were found to be thickened and fibrous over the entire brain, with a considerable increase of clear fluid between them. The brain was symmetric but small throughout, weighing 960 Gm. Other than this, nothing unusual was seen on gross sectioning of the cerebrum, cerebellum and pons. All of the ventricles were slightly dilated and filled with clear fluid.

From 3 to 4 per cent of the Purkinje cells in the cerebellum were found to be binucleate. The proportion of binucleate cells to the total number was as great as in the cerebellum of the senile mouse. The majority of the binucleate cells appeared in other respects very similar to the uninucleate cells in this specimen. They possessed as large an amount of Nissl substance as the other cells, the two nuclei were clear, the two nucleoli stained intensely, and in many instances a cap of basophilic substance was seen on each nucleus. For the most part the nuclei occupied a position near the center of the cell. They were almost invariably located opposite or very nearly opposite each other in the transverse diameter of the cell, and the nucleoli, in the center of each nucleus, lay thus in almost a straight line across the cell at its greatest transverse diameter (*2* in the figure).

The Da Fano preparations showed an interesting feature of the dendrites of the binucleate cells. In several instances the primary dendrite stem was double as far down as the cell body itself, and in some there was a considerable space between the sites of origin of the two dendrite trunks from the cell body. This feature was similar to that seen by silver impregnation of binucleate cells in senile mice;[6] here, as in the mouse, it may be the result of some abortive effort of the cell body, as well as of the nucleus, to divide.

The findings in the Nissl material, nuclei and nucleoli of the cells in the specimens from this group agree, in general, with those made in the 34 cerebellums previously studied. In the specimen from the 5 month old Negro the Nissl substance was not abundant and was either diffuse or in very small, powdery particles, as seen with the oil immersion lens. In that from the 13 month old Negro the Nissl substance was in the form of very small but readily distinguishable bodies, in some cells having a semblance of concentric arrangement about the nucleus and at the cell periphery. In the specimens from the 22 year old Negress, the 27 year old white man and the 49 year old Negress the Nissl bodies were of larger size, 3 to 4 microns in diameter, and in the specimens from the latter 2 persons they had a definite concentric arrangement. In the cerebellum of the 22 year old Negress the regular arrangement was lacking in many of the cells. In these 3 specimens the nuclei were clear and the nucleoli stained intensely. In



EXPLANATION OF FIGURE

1, a Purkinje cell (right), with a nucleus in process of amitotic division, from the cerebellum of a senile mouse, 746 days of age. The nucleus is of the dumbbell type, with a very definite constriction in the middle. A nucleolus is seen in the left half of the nucleus, but the nucleolus in the right half is not shown in this section. × 1,000.

2, a binucleate Purkinje cell from the cerebellum of a 22 year old Negress (cause of death: cerebrospinal syphilis). The cell is somewhat larger than the uninucleate cells in this subject but otherwise is similar to them, and both of the nuclei appear normal. × 750.

the cerebellum of the 56 year old white man many of the cells were hypochromatic, containing little or no basophilic material in the cytoplasm, the outlines of many of the cells were shrunken, the nuclei basophilic and the nucleoli pale.

COMMENT

In the cerebellum from a 22 year old Negress with cerebrospinal syphilis binucleate Purkinje cells were found and in rather high proportion. The cells containing two nuclei did not show visible evidence of any more degenerative condition than the uninucleate cells. I believe, however, that the binucleate condition had been brought about by the less favorable environment for the nerve cells caused by the disease and that it is evidence that the nuclei of human Purkinje cells, like those of the corresponding cells of mice and rats, will divide under unfavorable circumstances, although these circumstances apparently differ in degree or kind in rodents and in man. I believe, also, that evidence of an abortive attempt at cytoplasmic division can be seen in the wide separation of the two primary dendrite stems of many binucleate cells in the mouse and in man.

In regard to the question as to whether binucleate nerve cells may be brought about in any way other than by amitotic division, I may say that the answer obtained from all of the studies on binucleate Purkinje cells has been in the negative. According to Roussy and Mosinger,[8] plurinuclear neurons in the vegetative nuclei of the hypothalamus in mammals may arise as a result of fusion of uninucleate cells: *Certaines cellules bi et plurinucléées résultent manifestement, d'après nos constatations, d'une fusion d'éléments primitivement isolés.* (Certain binuclear and plurinuclear cells, result manifestly, according to our understanding, from a fusion of elements that originally were isolated.) They also stated, however:[9] *D'autres fois la plurinucléose est due à une division nucléaire amitotique, et généralement inégale.* (In other instances the plurality of nuclei is due to an amitotic and generally unequal nuclear division.) All available evidence on the cause of the binucleate condition in the Purkinje cells is in favor of amitotic division; in many cases single nuclei with two nucleoli are seen, and nuclei of dumbbell shape with a nucleolus in each expanded portion and a greater or less constriction in the middle are common (*1* in the figure). The amitotic division in the Purkinje cell seems usually to divide the nucleus into almost equal halves, and each half becomes a normal nucleus. Roussy said of the amitotic division in the cells of the hypothalamus: *Elle s'opère, en effet, le plus souvent, par bourgeonnement. Ainsi s'expliquent les cellules*

8. Roussy, G., and Mosinger, M.: Compt. rend. Soc. de biol. **118**:736, 1935.

9. Roussy and Mosinger,[8] p. 737.

binucléées dont le second noyau, sans signe de dégénérescence, est de petite taille, et dépourvu de nucléole. (It takes place most frequently by budding. This explains the binucleate cells in which, though there is no evidence of degeneration, the second nucleus is small and does not have a nucleolus.) This *bourgeonnement,* or budding, of the nuclei appears to be a very rare occurrence in the nuclei of the Purkinje cells, in which equal binary fission is the usual type of division.

SUMMARY

Binucleate cells have been found in considerable numbers (3 to 4 per cent of the total number of cells) in the cerebellum of a 22 year old Negress who died of cerebrospinal syphilis. This is the only specimen showing such cells out of 40 specimens of human autopsy material which have been examined by the author.

The binucleate condition is the result of amitotic division of the nucleus into two equal or almost equal parts. Nuclear division is preceded by division of the nucleolus.

In some instances there appears to be an abortive attempt at cytoplasmic division of the Purkinje cell, as evidenced by the separation of the dendrite trunks at their origin from the cell body.

The binucleate condition of the Purkinje cell and the mode of division by which it is brought about seem to be essentially similar in man and in rats and mice. The factors bringing about the division may, however, be different in degree or kind, since up to the present binucleate cells have been found in man only in pathologic states, while in rodents they occur as a feature of normal senility.

The present findings on age changes in the Nissl material, nucleus and nucleolus of the Purkinje cells of 6 persons ranging in age from 5 months to 56 years are in agreement with earlier work on the Purkinje cell in man.

CARDIAC LESIONS IN RABBITS PRODUCED BY A FILTRABLE VIRUS (VIRUS III)

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Recently during the course of some studies [1] on the prevalence of virus III in rabbit breeding stocks it was observed that in animals inoculated with this virus cardiac lesions occasionally developed which could be attributed directly to the infection. This occurrence is of especial interest in that it is the first example of spontaneous disease of the heart in which the presence of a filtrable infectious agent in the lesions in the myocardium, endocardium and pericardium can be definitely proved by the observation of inclusion bodies, a characteristic and pathognomonic histologic change visible under the microscope. The following report contains a description of these lesions and of several methods which have been successful in increasing the incidence of virus localization in the heart after peripheral inoculation.

Virus III has been known since 1923, when Rivers and Tillett [2] and independently shortly afterward Miller, Andrewes and Swift [3] isolated it from presumably normal rabbits by serial testicular passage. A year later McCartney [4] found the same virus in rabbits in England, and in 1926 Doerr [5] reported the disease from Switzerland.

The incidence of spontaneous infection in rabbit breeding stocks is apparently low. Rivers estimated that 15 to 20 per cent of stock rabbits were refractory to infection, presumably because of a preceding attack of the naturally acquired disease. These figures agree very well with those of Topacio and Hyde,[6] who found that 17 per cent of 76 Maryland rabbits which they tested in 1931 could not be infected. The refractory state was most often seen in the older animals. On the other hand, of 377 rabbits inoculated in England by Andrewes [7] in 1928, 369, or 97.7 per cent, were susceptible, and in 1938 an investigation [1] of the

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1. Pearce, J. M.: Proc. Soc. Exper. Biol. & Med. **38**:872, 1938.
2. Rivers, T. M., and Tillett, W. S.: J. Exper. Med. **38**:673, 1923.
3. Miller, C. P., Jr.; Andrewes, C. H., and Swift, H. F.: J. Exper. Med. **40**: 773, 1924.
4. McCartney, J. E.: Annual Report of the Metropolitan Asylums Board, London, 1924-1925, p. 152.
5. Doerr, R.: Zentralbl. f. Bakt. (Abt. 1) **97**:76, 1926.
6. Topacio, F., and Hyde, R. R.: Am. J. Hyg. **15**:99, 1932.
7. Andrewes, C. H.: J. Path. & Bact. **31**:461, 1928.

incidence of the disease in several breeding stocks in the vicinity of New York city failed to show any evidence of infection as determined by neutralizing antibodies in the serums of 163 sample animals from various colonies the total population of which was 12,000. In addition, all of 90 rabbits from the largest single colony on being inoculated with potent virus showed typical lesions.

No species other than the rabbit has as yet been shown to be susceptible, and nothing is known of the pathologic nature or method of transmission of the naturally occurring disease. In animals inoculated in the laboratory as a routine lesions develop at the sites of inoculation which are characterized histologically by typical large acidophilic intranuclear inclusion bodies around which there is a clear zone or halo and peripheral margination of chromatin. These bodies may be found in a variety of cells. For routine work testicular inoculation has been the method of choice, and in this organ inclusions are most numerous in the interstitial cells, fibroblasts and large mononuclear phagocytes. They may sometimes be found in endothelial cells in the epithelium of the rete and tubuli recti. Cowdry [8] described them also in spermatogonia and spermatocytes. Inoculation of the scarified cornea results in the formation of inclusions in the corneal epithelium. Similar bodies are seen in the epidermis following intradermal inoculation. Recently Rivers and Stewart [9] demonstrated the same intranuclear inclusions in nerve cells and glia cells in the encephalitis produced by injecting the virus into the brain. Instillation of infective material into the nostrils of rabbits under ether anesthesia brings about mild pneumonia with an exudate composed of large mononuclear phagocytic cells, which frequently contain inclusions.

Rivers [2] showed that the virus will localize and produce lesions in scarified skin when injected intravenously, but with this one exception, regardless of the route of entrance and in spite of the fact that the virus must enter the blood, since the latter is infectious during the acute stage of the disease, lesions have never been reported in any regions or organs other than those directly inoculated.

In the present work the first virus III lesion of the heart was seen in a rabbit which had been bled with needle and syringe from the heart in the routine collection of normal serum immediately before intratesticular inoculation of the virus. Soon after this similar lesions were discovered in an animal which had had no previous treatment or trauma other than that entailed in the inoculation of the virus. The experiments reported here were then begun in order to study this phenomenon further. It was found that a much greater incidence and severity of cardiac damage could be brought about by subjecting the rabbit to a large intravenous injection of acacia at the time of inoculation or by

8. Cowdry, E. V.: Arch. Path. **10**:23, 1930.
9. Rivers, T. M., and Stewart, F. W.: J. Exper. Med. **48**:603, 1938.

repeated small doses of pitressin (betahypophamine), as well as by the more drastic method of cardiac puncture.

A total of 75 animals have been inoculated by either the intratesticular or the intravenous route with material proved to contain active virus. These have been divided into four groups: (1) rabbits given acacia intravenously at the time of inoculation with virus III, (2) rabbits whose hearts were punctured for bleeding just before the peripheral inoculation of virus III, (3) rabbits given one or more doses of pitressin either at the time of the inoculation of virus III or during the course of the infection, and (4) rabbits inoculated with virus III with no other treatment. Although the lesions in all four of these groups were fundamentally the same, they varied so considerably in incidence, severity and localization that the findings in each have been considered separately.

METHOD AND MATERIAL

All the rabbits were young males weighing between 1,500 and 3,000 Gm., the majority being around 2,500 Gm. No attempt was made to select them according to breed or color, and they were obtained from several different sources. The inoculum was either the supernatant fluid of a saline suspension of infected rabbit testis or the fluid portion of a tissue culture infected with virus III. Although filtration was not done, gross bacterial contamination was ruled out by culturing the material in the usual broth or agar mediums. The ease with which virus III will multiply in vitro in tissue culture has been amply demonstrated by Andrewes [10] and in this laboratory.[11] The method of cultivation is described in detail elsewhere.[11] Tissue cultures provide an excellent source of material containing active virus in liquid form for intravenous injection, since the fluid portion is free from gross particles, which might cause embolization, and contains none of the toxic substance [12] found in tissue suspensions or extracts. Culture fluid was used undiluted.

Testis suspensions were prepared by grinding a testis removed aseptically from an animal killed on the fourth or fifth day after intratesticular inoculation in a mortar with sterile sand and 0.9 per cent salt solution. As a rule 20 cc. of saline solution was used for each testis, but occasionally the suspension was made with 10 or 30 cc. Since virus III is never lethal and often produces only a very mild reaction both clinically and histologically, it seemed advisable to use as concentrated an inoculum as possible. Experimental animals were given an injection of 0.5 to 2 cc. of either suspension or culture fluid in each testis, or an injection of 2 to 15 cc. of culture fluid in the marginal ear vein. Occasionally both routes were employed. Two were inoculated by dropping 2 cc. of virus material into each nostril with the animal under ether anesthesia.

Acacia solution was prepared by dissolving the dried powder (Squibb) in warm 0.9 per cent saline solution, in amounts sufficient to make concentrations of either 10 or 20 per cent, and was sterilized by immersion, in a cotton-plugged flask, in boiling water for one hour. Following this, no growth occurred when it was

10. Andrewes, C. H.: Brit. J. Exper. Path. **10**:188, 1929.

11. Pearce, J. M.: J. Immunol., to be published.

12. When a filtrate from a suspension of infected testis is injected intravenously, even in doses as small as 0.05 cc., death occurs almost instantaneously. This effect can be avoided by sufficient dilution.

cultured on the usual bacterial mediums. The experimental animals received a single injection either immediately before or immediately after the inoculation of the virus. Just before use the acacia was warmed to approximately 38 C. and then introduced slowly into the marginal ear vein in a dose varying from 25 to 65 cc., the heavier animals getting the larger amounts.

Cardiac puncture was performed by the usual technic used in bleeding, and 10 to 20 cc. of blood was withdrawn to make certain that the needle actually had pierced the myocardium. While the rabbit was still supine on the board, the virus material was injected into the testes or marginal ear vein, but immediately thereafter it was released, and following a short period of observation to rule out any gross cardiac damage from the puncture it was returned to its cage.

The group given pitressin received the drug intravenously in a single injection of 0.5 cc. at the time of the inoculation of virus or in several doses variously spaced throughout the experimental period. The preparation used was pitressin (betahypophamine) put up by Parke, Davis and Company in 1 cc. sterile glass ampules.

Three to six days after infection the experimental animals of all groups were killed and autopsies made. The hearts were examined externally but were not opened, since it was found that more satisfactory sections for histologic study could be obtained by fixing the intact organ and later trimming it in such a way as to include all four chambers and one or more valves in their usual relationships. By cutting several parallel blocks from a single heart it is not difficult to show at least three of the valves and often four without resorting to serial section. Zenker fixation was used throughout, and paraffin sections were stained with hematoxylin and eosin and with Giemsa. In the great majority of instances histologic examination also included the lungs, spleen, liver, adrenals, kidneys and testes, while occasionally the pancreas, thyroid, thymus or salivary gland was studied.

RESULTS

Since the most constant, widespread and severe lesions occurred in animals prepared by intravenous injection of acacia at the time of the inoculation of virus III, the findings in this group are described first and in greater detail, while the results obtained in the other three groups are presented only so far as they differ or fall short of this more extreme picture.

Rabbits Given Acacia Intravenously at the Time of Virus III Inoculation.—Acacia in varying doses was injected intravenously into 22 animals, and 17 of these were also inoculated with material containing active virus. The remaining 5 served to control the effects of acacia alone. As shown in table 1, this method was highly successful. Sixteen of the 17 experimental animals, or roughly 94 per cent, showed severe cardiac lesions, in which typical inclusion bodies were easily demonstrated, while the remaining one showed some evidence of cardiac involvement although inclusions could not be seen. There were no noteworthy changes in the hearts or other organs of any of the 5 controls.

In the affected animals the predominant lesion was myocarditis, which was distributed equally between auricles and ventricles, but which was much more severe on the right side of the heart and was sometimes confined to it entirely. Figure 1 shows an extreme example of

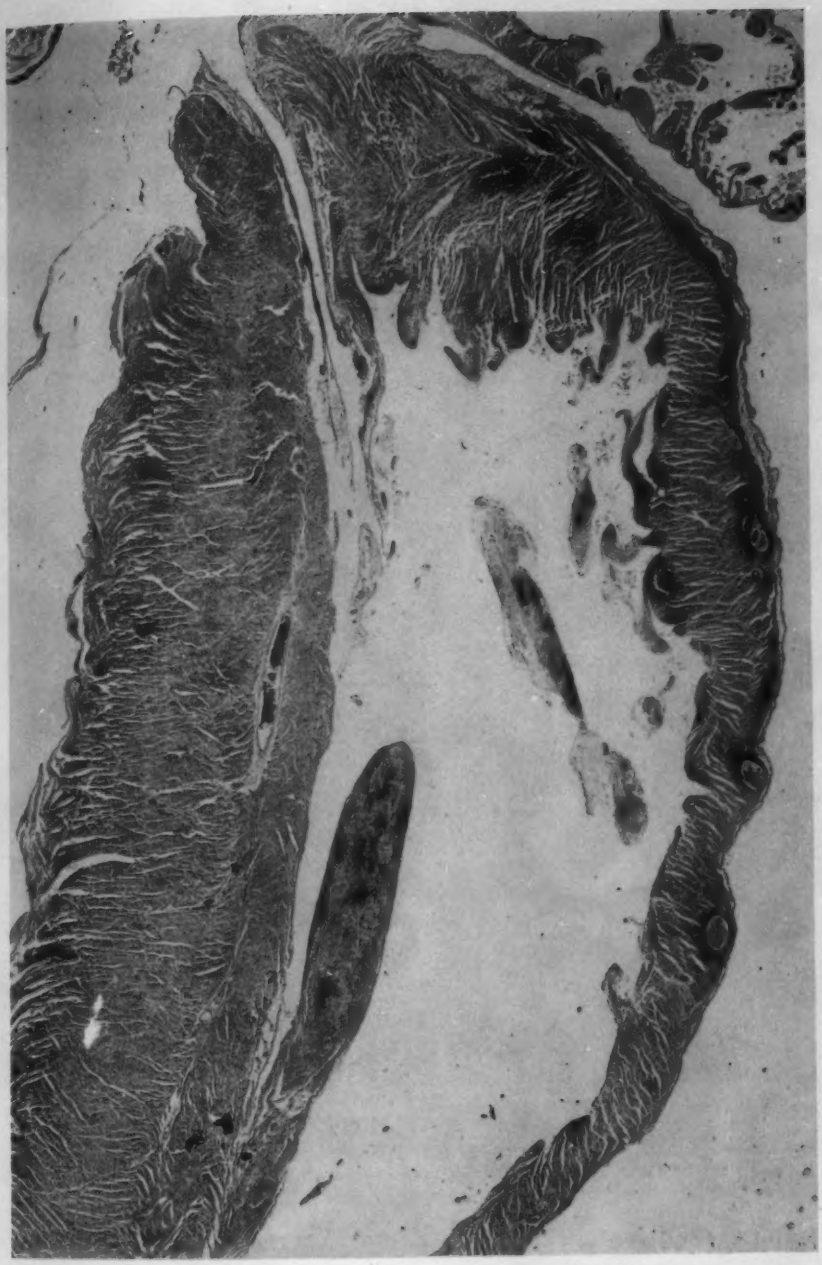

Fig. 1 (rabbit 501, killed four days after intratesticular injection of virus III and intravenous injection of acacia).—Inflammatory foci in the wall of the right ventricle and in the papillary muscle. Paravascular localization is evident. Hematoxylin and eosin; × 10.

this tendency to affect the right ventricular wall more than the left. It also brings out the manner in which lesions tended to occur around or in the vicinity of blood vessels, either veins or arteries. The lesions in the myocardium in their milder form consisted of nodular collections of large mononuclear cells and lymphocytes together with some fibroblastic proliferation. Only rarely were polymorphonuclear leukocytes

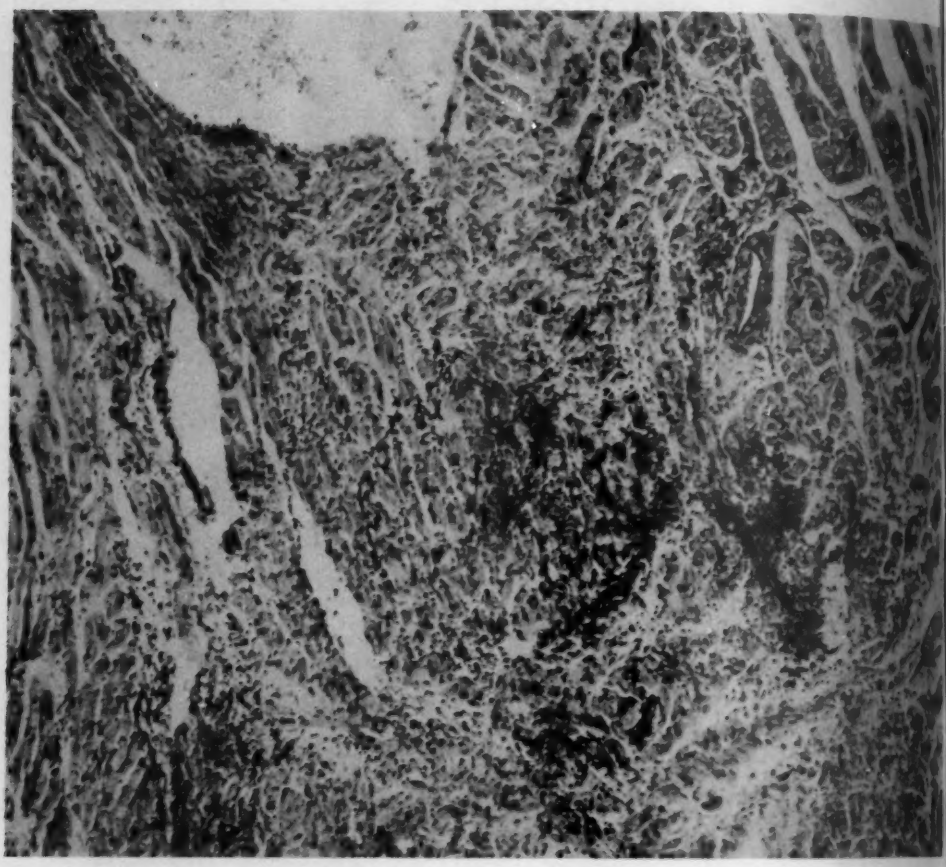

Fig. 2 (rabbit 445, killed five days after intratesticular and intravenous injection of virus III and acacia, respectively).—Areas of infiltration and hemorrhage in the wall of the right auricle. Hematoxylin and eosin; × 100.

seen. In the areas more markedly affected a variable amount of necrosis of muscle was seen, there was more new growth of fibrous tissue, and a much greater number and density of mononuclears occurred; if the lesion bordered on a blood vessel, swelling and necrosis of the perivascular collagen sometimes took place. Occasionally there was some

subendothelial inflammatory infiltration in veins, but the walls of arteries and arterioles never showed any definite change. Rarely the inflammation was more diffuse, infiltrating cells being spread throughout a large

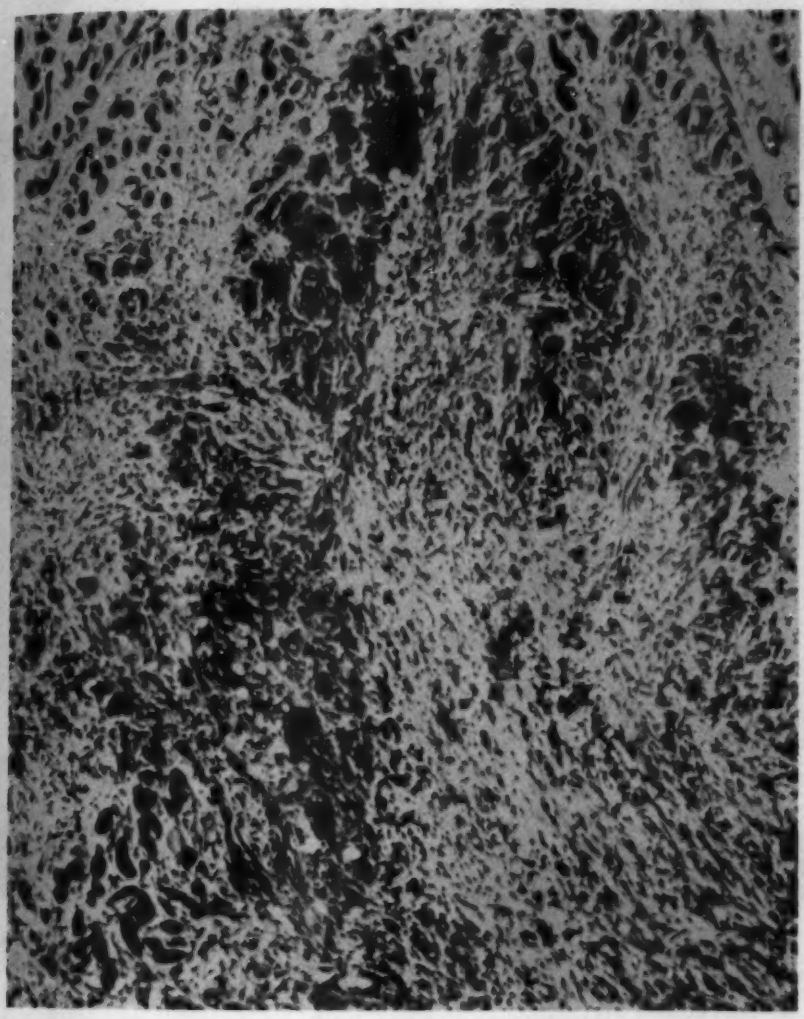

Fig. 3 (rabbit 142, killed five days after cardiac puncture and intravenous injection of virus III).—Large area of necrosis and infiltration in the wall of the left ventricle. Hematoxylin and eosin; × 130.

area of myocardium with few dense nodular aggregates. In several hearts material resembling calcium both by its appearance in sections stained with hematoxylin and eosin and by the von Kossa reaction was deposited in the necrotic muscle fibers. Typical intranuclear inclusion

bodies occurred in the infiltrating mononuclear cells, in fibroblasts and less frequently in cardiac muscle cells. In the more severe lesions one sometimes found large giant cells with two or more closely crowded, centrally placed round nuclei and indefinitely outlined basophilic cytoplasm. Although these cells were remarkably similar to the Aschoff cells of rheumatic fever, it seems more probable that they were degenerat-

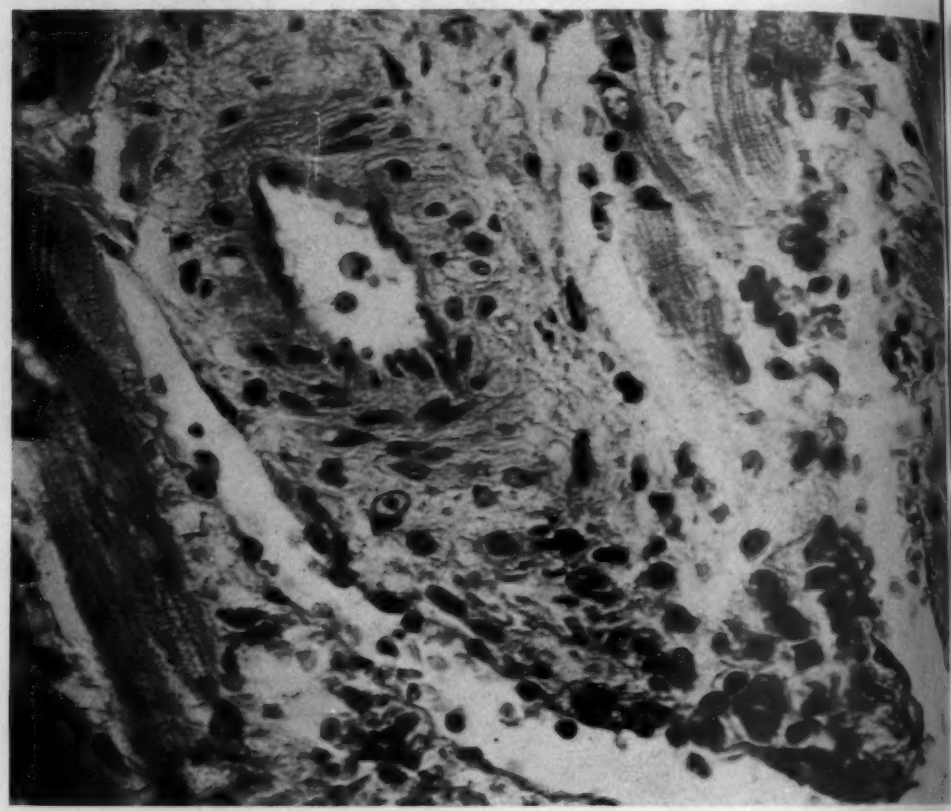

Fig. 4 (rabbit 219, killed four days after intravenous injection of acacia and intravenous and intratesticular injection of virus III).—Inclusion bodies in inflammatory cells and fibroblasts near an arteriole and beneath the endocardium of the right auricle. Hematoxylin and eosin; × 450.

ing muscle fibers. These are shown in figure 7. Figures 2 to 5 illustrate various types of the myocardial reaction and the inclusions constantly associated with them.

Lesions of the endocardium, although common, were less constant. A varying degree of inflammatory reaction in localized areas on and just beneath the lining membrane of the cardiac chambers was observed

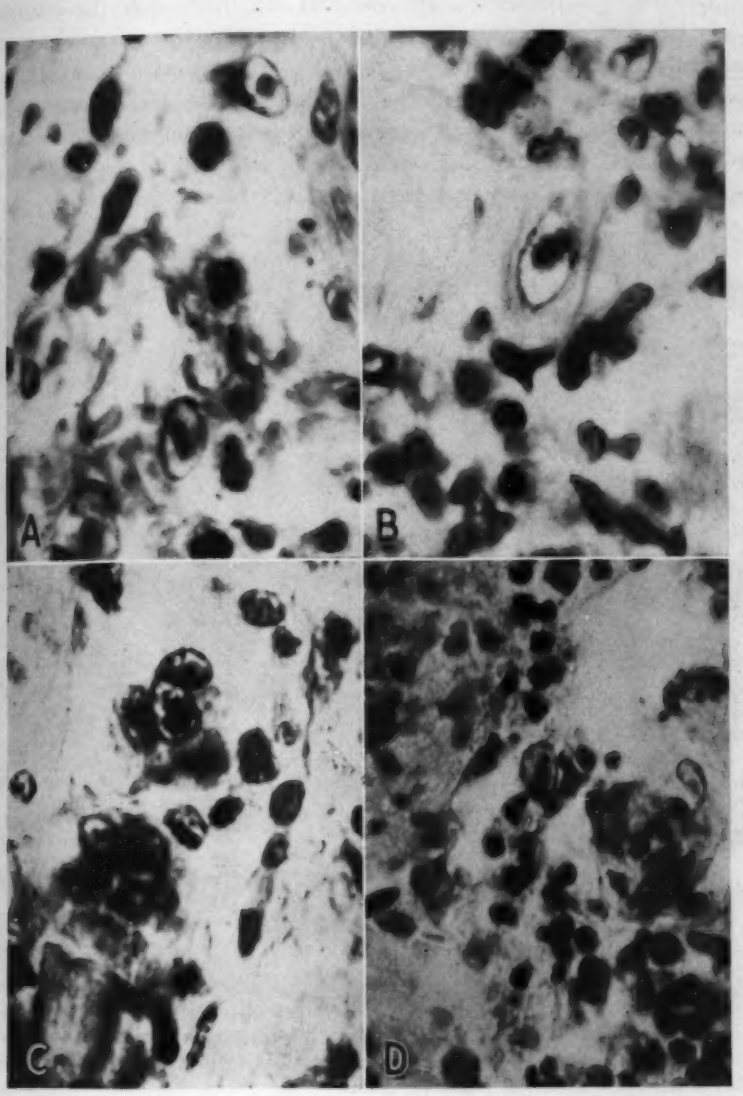

Fig. 5.—*A*, part of the lesion reproduced in figure 3, showing intranuclear inclusion bodies in an infiltrated area. Hematoxylin and eosin; × 750. *B*, part of the lesion reproduced in figure 3, showing an intranuclear inclusion body in a heart muscle cell. Hematoxylin and eosin; × 1,040. *C* (rabbit 501), two multinucleated cells, resembling Aschoff cells, lying near a necrotic muscle fiber in an inflammatory area. Hematoxylin and eosin; × 1,000. *D* (rabbit 445), part of the lesion reproduced in figure 2, showing inclusion bodies in inflammatory cells near a venule. Hematoxylin and eosin; × 550.

in 13 of the 17 experimental animals, and in 7 of these 13 the substance of one or more of the valves was also infiltrated with mononuclear cells, frequently containing inclusions (fig. 6). True vegetations on the surface of the valves were never found, although the leaflets were often thickened, and mitotic figures were not uncommon among the proliferating fibroblasts. Small hemorrhages into the substance of the valves were common, but these have not been considered as caused by the virus

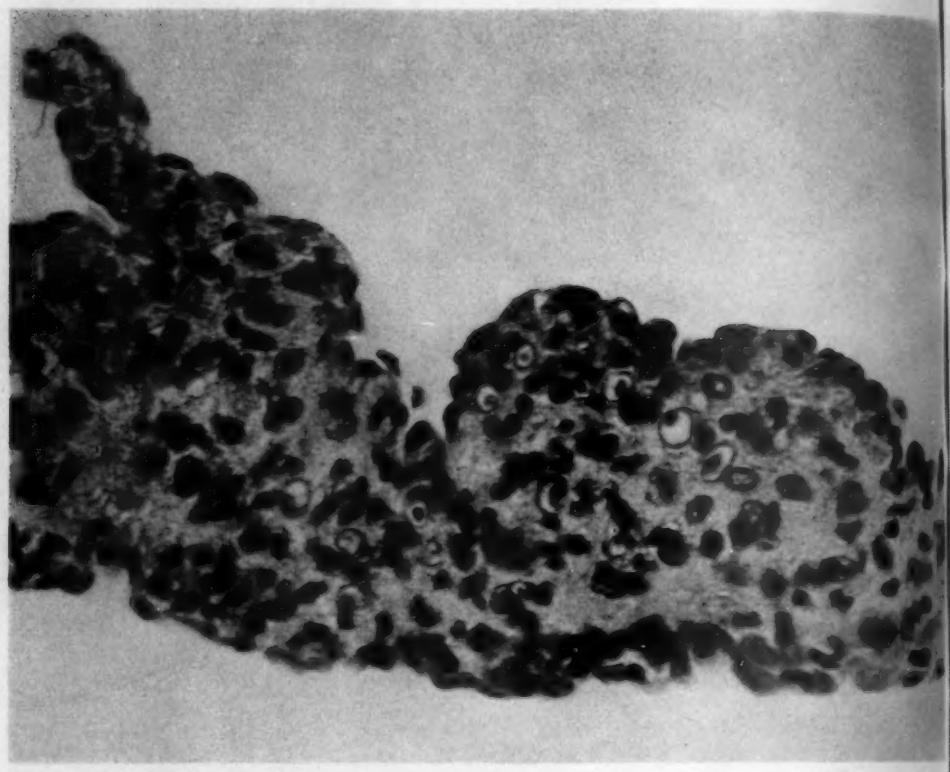

Fig. 6 (rabbit 507, killed six days after intravenous injection of acacia and intratesticular and intravenous injection of virus III).—Infiltration and fibroblastic proliferation with numerous inclusion bodies in the tricuspid valve. Hematoxylin and eosin; × 450.

unless inclusion bodies were also present. At times the chordae tendineae showed diffuse or nodular thickening and infiltration similar to that in the valve proper. In the animals treated with acacia the mural endocarditis was never extensive, consisting solely of plaquelike collections of mononuclear cells and lymphocytes, again with occasional inclusion-bearing nuclei.

Evidence of pericardial involvement was found in only 2 animals in this group. It consisted of a delicate coating of fibrin over the upper part of the ventricle, in the auriculoventricular sulcus and in the crevasses of the auricular surfaces. The mesothelial layer beneath this remained intact in many places, but the cells composing it appeared enlarged. Rarely they contained intranuclear inclusions.

Localization of the virus in organs other than the heart and the testis that was directly inoculated occurred in 4 animals. One rabbit had a

TABLE 1.—*Rabbits Given Acacia Intravenously at the Time of Inoculation with Virus III*

Number of experimental animals	17
Number having cardiac lesions with inclusion bodies	16
Percentage of animals showing positive results	94.1
Number of control animals given acacia alone	5
Number of control animals with cardiac lesions	0

Distribution of Cardiac Lesions in Animals with Positive Findings

Rabbit	Virus III Inoculum, Amount and Route	Amount of Intravenous Acacia, Cc.	Days After Inoculation	Myocarditis	Endocarditis: Mural	Endocarditis: Valvular	Pericarditis
210	Culture fluid, 15 cc., I.V.*	25	6	+++	++	+	+
220	Culture fluid, 10 cc., I.V.	65	4	+++	++	0	++
222	Culture fluid, 15 cc., I.V.	45	4	++++	+++	+++	0
486	Testis susp., 3 cc., I.T.†	50	4	++	0	0	0
488	Testis susp., 2.5 cc., I.T.	40	4	+	0	0	0
491	Testis susp., 3 cc., I.T.	30	6	++	0	0	0
492	Testis susp., 2 cc., I.T.	45	4	+++	++	0	0
501	Testis susp., 2 cc., I.T.	50	4	++++	++	++	0
219	Culture fluid, 10 cc., I.V., 2 cc., I.T.	45	4	+++	+	0	0
442	Culture fluid, 15 cc., I.V., 2 cc., I.T.	55	4	++++	++	++	0
443	Culture fluid, 7 cc., I.V., 2 cc., I.T.	55	4	+++	++	0	0
444	Culture fluid, 15 cc., I.V., 2 cc., I.T.	65	5	++	+	0	0
445	Culture fluid, 15 cc., I.V., 2.5 cc., I.T.	60	5	++++	+++	0	0
475	Testis susp., 2 cc., I.T., 2 cc., I.V.	50	3	++++	+	++	0
506	Testis susp., 3 cc., I.T., 1 cc., I.V.	50	4	+++	++	+	0
507	Testis susp., 3 cc., I.T., 2.5 cc., I.V.	50	6	++++	+	++++	0

* Intravenous.
† Intratesticular.

small necrotic and inflammatory focus containing virus III inclusions in the liver, and 3 others had tiny similar lesions in the adrenals.

Rabbits Given an Injection of Virus III Following Cardiac Puncture.—The incidence of cardiac involvement in this group did not differ significantly from that in the acacia group. The findings are presented in table 2. Again the lesions were severe and myocarditis was predominant, but the ventricles suffered much more than the auricles, and the left ventricle was more frequently and more severely damaged than the right. In histologic detail the myocardial lesions were identical with those just described. Seldom were they grouped around the probable path of the needle, and often wide areas of necrosis and infiltration spread through the muscular wall (fig. 3), apparently originating

from or being more intense in the neighborhood of a small blood vessel. Calcification of necrotic muscle was again a not infrequent finding.

The valves only occasionally showed any definite change, but mural endocarditis of a greater or less extent was almost constant. In the more extreme examples of this process thrombi were attached to the affected area and necrosis of muscle extended for an appreciable distance into the cardiac wall. Figure 7 illustrates one of these more advanced

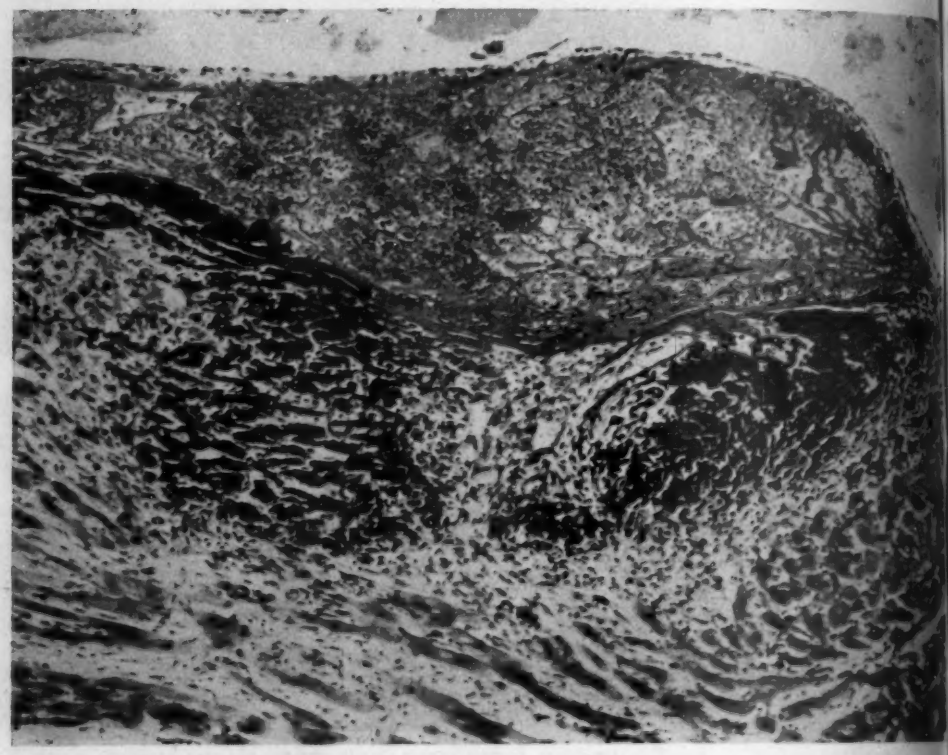

Fig. 7 (rabbit 154, killed four days after cardiac puncture and intratesticular injection of virus III).—Thrombus adherent to an infiltrated and necrotic area on the endocardial surface of the left ventricle. The necrotic muscle fibers here contain calcium. Hematoxylin and eosin; × 100.

lesions, which may well represent the point of entrance of the needle into the ventricular chamber. However, such lesions did not result from puncture alone, and furthermore inclusions were present in the exudate.

In 5 of the 15 animals extensive fibrinous pericarditis was observed. This consisted of a shaggy yellow coat of fibrin, visible grossly and most abundant at the base of the heart and over the auricles. Microscopically a layer of the typical mononuclear inflammatory cells was seen to lie beneath the fibrin, extending here and there into the muscle for a short

distance (fig. 8). These cells and the nuclei of plump basophilic cells, resembling desquamated mesothelium caught in the meshes of the fibrin, contained inclusions.

Extracardiac inclusions, aside from those in the region directly inoculated, occurred in only 1 animal, in which they were found in the spleen.

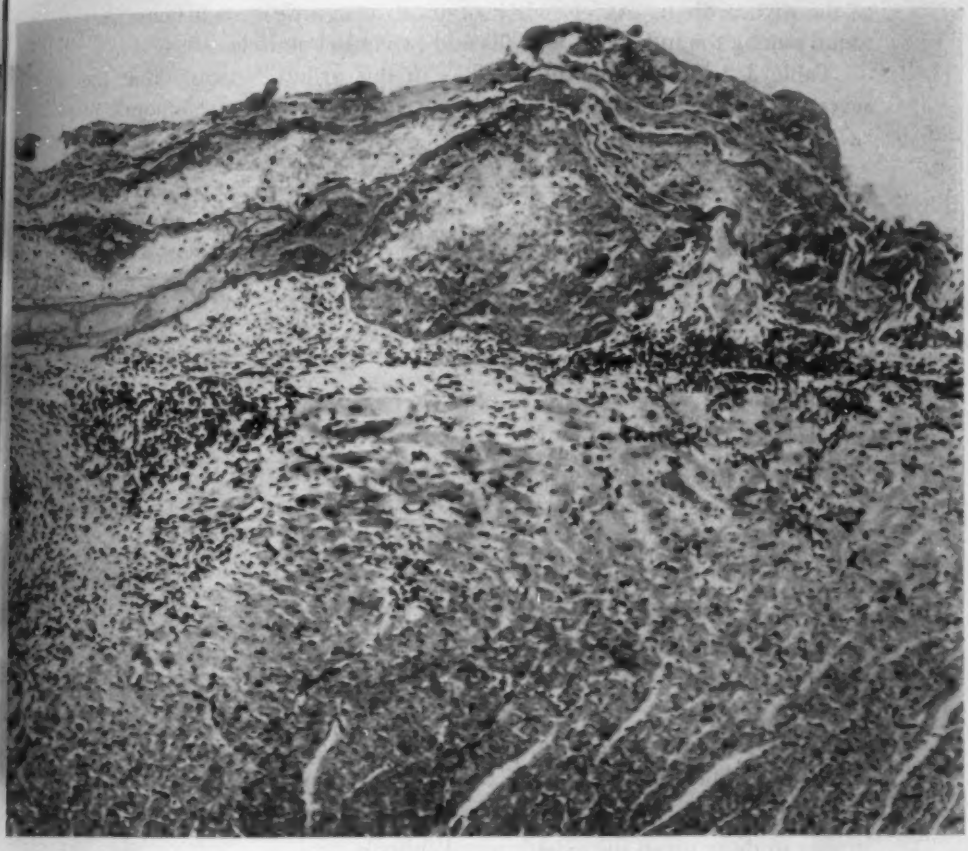

Fig. 8 (rabbit 142, killed five days after cardiac puncture and intravenous injection of virus III).—Pericarditis and cellular infiltration of the underlying myocardium. Hematoxylin and eosin; × 100.

Rabbits Given Pitressin Preceding or During Virus III Infection.—This method was not as successful as the other two in producing cardiac localization of the virus, but well marked lesions, accompanied by inclusions, developed in the hearts of 6 of the 13 inoculated animals, while in 2 others there were less extensive lesions without inclusions. With

the exception of the directly inoculated testis, no other organs were affected.

The affinity of the virus for the muscle of the left ventricle was even more marked here than in the cardiac puncture group. In fact, no significant changes were seen elsewhere than in this region. The inflammatory reaction was not as acute or localized, and the necrotic element was slight, but diffuse areas of infiltration and fibrosis occupied much of the myocardium. As elsewhere, typical intranuclear inclusions were found among the inflammatory cells and rarely in a muscle cell.

Table 3, which shows the findings in this group, suggests that the severity of the lesion may depend to some extent on the amount of

TABLE 2.—*Rabbits Infected with Virus III Following Cardiac Puncture*

Number of experimental animals	15
Number having cardiac lesions with inclusion bodies	13
Percentage of animals showing positive results	86.7

Distribution of Cardiac Lesions in Animals with Positive Findings

Rabbit	Virus III Inoculum, Amount and Route	Days After Inoculation	Myocarditis	Endocarditis		Pericarditis
				Mural	Valvular	
128	Testis susp., 2 cc., I.T.*	5	++++	+++	+	0
135	Testis susp., 2 cc., I.T.	3	++++	++	0	++++
140	Testis susp., 2 cc., I.T.	3	+++	0	0	0
150	Testis susp., 2 cc., I.T.	4	+++	+	++	0
154	Culture fluid, 2 cc., I.T.	4	++	+++	0	0
173	Culture fluid, 2 cc., I.T.	4	++++	+	0	++++
179	Testis susp., 2 cc., I.T.	3	++	0	0	0
414	Testis susp., 3 cc., I.T.	3	+++	++	+++	+++
415	Testis susp., 3 cc., I.T.	4	+	+	0	0
432	Testis susp., 3 cc., I.T.	3	+++	+	0	0
191	Culture fluid, 4 cc., I.N.†	4	++++	+	0	0
193	Culture fluid, 4 cc., I.N.	4	++++	++	0	++++
142	Culture fluid, 3 cc., I.V ‡	5	++	+	0	++++

* Intratesticular.
† Intranasal.
‡ Intravenous.

pitressin, but that pitressin alone is responsible for the myocardial reaction seems improbable in view of the absence of any lesion in the 5 controls, all of which received the drug in amounts either in excess of or similar to those given the experimental animals.

Rabbits Given No Treatment Other Than Inoculation with Virus III.—Of 20 animals inoculated with virus III by the intratesticular or the intravenous route, 5 showed cardiac lesions with inclusion bodies. These lesions were similar in appearance and distribution to those seen in the acacia group but never reached comparable degrees of severity. As a rule, they were small focal areas of infiltration near or alongside arterioles in the musculature of the ventricles and auricles, while occa-

sionally an equally mild but diffuse inflammatory reaction spread throughout a large area of myocardium. Collections of lymphocytes and mononuclear cells sometimes lay in and just beneath the endocardium, but these were seldom large or prominent. One heart had a delicate coating of fibrin over the epicardial surface of the auricles.

TABLE 3.—*Rabbits Given Pitressin Preceding or During Virus III Infection*

Number of experimental animals	13
Number having cardiac lesions with inclusion bodies	6
Percentage of animals showing positive results	46.2
Number of control animals given pitressin alone	5
Number with cardiac lesions	0

Distribution of Cardiac Lesions in Animals with Positive Findings

Rabbit	Virus III Inoculum, Amount and Route	0.5 Cc. Doses of Pitressin	Days After Inoculation	Myocarditis	Endocarditis: Mural	Endocarditis: Valvular	Pericarditis
434	Testis susp., 2 cc., I.T.*	8	4	++++	+	0	0
440	Testis susp., 4 cc., I.T.	6	4	++++	+	++	0
484	Testis susp., 2 cc., I.T.	1 on first day	4	+	0	0	0
498	Testis susp., 3 cc., I.T.	4	4	++++	+	0	0
510	Testis susp., 4 cc., I.T.	3	3	++	+	0	0
513	Testis susp., 2.5 cc., I.T.	4	4	+++	0	0	0

* Intratesticular.

TABLE 4.—*Rabbits Given No Treatment Other Than Inoculation with Virus III*

Number of animals in experiment	20
Number having cardiac lesions with inclusion bodies	5
Percentage of animals showing positive results	25

Distribution of Cardiac Lesions in Animals with Positive Findings

Rabbit	Virus III Inoculum, Amount and Route	Days After Inoculation	Myocarditis	Endocarditis: Mural	Endocarditis: Valvular	Pericarditis
205	Culture fluid, 10 cc., I.V.*	6	++	+++	0	0
206	Culture fluid, 10 cc., I.V.	5	+	+	+	+
225	Culture fluid, 11 cc., I.V.	5	+	+	0	0
114	Testis susp., 1 cc., I.T.†	6	+	0	+	0
406	Testis susp., 2 cc., I.T.	3	++	0	+	0

* Intravenous.
† Intratesticular.

In 1 rabbit inclusion bodies were found in the peribronchial lymphoid tissue in the lung, and in 3 others they were present in the adrenals. No other extracardiac localization was observed.

COMMENT

The demonstration of a filtrable virus as the causative agent in a form of heart disease in the rabbit is of interest in several connections. Of practical importance is the fact that since virus III is at times a

spontaneous infection in rabbit colonies the possibility of its presence must be taken into account in interpreting the results of experimental work dealing with cardiac disease.

Of theoretic significance is the analogy with rheumatic heart disease. While it is by no means implied that virus III has any etiologic significance in rheumatic fever, the parallel between the lesions caused by it and those in the latter disease is obvious and adds some evidence in favor of the opinion that rheumatic fever, too, is caused by a filtrable infectious substance. In both conditions there is frequently "pancarditis." The fibrinous pericarditis is very similar. The myocardial lesions are the same in localization, and, although the specific Aschoff cell is seen only in rheumatism, the reacting cell type is much the same in both. Virus III disease does not have the vegetations on the valves, but the collagenous swelling and degeneration and the later proliferative changes are not unlike.

A search of the literature has revealed only one other report of the production of heart disease by peripheral inoculation of a filtrable virus with localization of the virus in the heart. Andrei and Ravenna[13] described "thrombo-endocarditis" occurring spontaneously in rabbits, which could be transmitted from animal to animal both by contact and by intraperitoneal injection of blood or endocardial vegetations and which they believe was caused by an ultramicroscopic agent. The lesions in their animals were quite unlike those seen in virus III infection; they consisted chiefly of large thrombotic vegetations adherent to the endocardium of the right side of the heart auricle, ventricle or tricuspid valve—with no associated inflammatory cells and no involvement of the myocardium. No inclusion bodies were mentioned. The virus III lesion is almost the exact opposite of this, being predominantly myocardial, essentially inflammatory, constantly containing inclusion-bearing cells and only rarely showing a true thrombotic vegetation. Andrei and Ravenna also considered the possibility of this virus being identical with virus III and ruled it out on immunologic as well as morphologic grounds.

In their early studies Miller, Andrewes and Swift [8] produced myocardial and pericardial lesions in rabbits by "intrathoracic" injections of virus III material but did not record examination of the hearts of animals inoculated at other sites. It is of interest, however, that at approximately the same time that Rivers estimated that 15 to 20 per cent of stock rabbits in the Rockefeller Institute laboratories were refractory to virus III because of previous spontaneous infection, Miller,[14] working in

13. Andrei, G., and Ravenna, P.: Arch. Int. Med. **62**:377, 1938.
14. Miller, C. P., Jr.: J. Exper. Med. **40**:543, 1924.

the same institution, described spontaneous myocarditis occurring in 20 of 34 presumably healthy rabbits which he examined. Although Miller specifically stated that no cell inclusion bodies were found, it is still possible that some at least of his lesions may have been caused by a previous or concomitant virus III infection, since the inclusions disappeared after six or seven days.

On the other hand, the probability that the lesions described in the present paper preceded the virus III infection and that the occurrence of this virus in them, as indicated by inclusion bodies, was only incidental seems unlikely. It is true that the myocardial lesions noted by Miller were very similar, save for the absence of inclusions, to those in the group receiving no treatment other than peripheral inoculation with virus III, and it cannot be denied that myocarditis of as yet unknown origin may have been already present and may have provided an inflammatory focus in which the circulating virus lodged. However, significant lesions were not found in the controls, and in the hearts prepared by acacia, pitressin or puncture the lesions containing cell inclusion bodies were so severe and widespread as to make it seem highly improbable that virus III was not at least the chief etiologic factor. It is important to emphasize again that in none of the hearts of the animals in any of the four groups of experiments reported here were the inflammatory changes considered as being caused by virus III unless the typical intranuclear inclusion bodies could be demonstrated in stained sections.

The mechanism of the methods used to increase the incidence of cardiac involvement remains obscure. The effect of puncture, as stated, was discovered accidentally during routine collection of serum. It is not difficult to conceive of any such comparatively gross damage to tissue determining the localization of circulating virus since it is well known that these substances do become concentrated in local areas of trauma or inflammation, and, in fact, Rivers and Tillett [2] demonstrated in 1923 that virus III would produce lesions in the scarified skin or cornea when injected intravenously.

A recent report by Nedzel,[15] in which he showed that intravenous administration of pitressin brought about localization of bacteria on the heart valves of dogs, with bacterial endocarditis resulting, led to the use of this drug in the hope that a similar phenomenon would bring about a virus-induced vegetation. Although no vegetation occurred, the myocardial lesions were increased. Nedzel explained his results on the basis of hypertension, the so-called "pressor episode," which in his opinion alters the endocardium and endothelium in such a way as to make it more receptive to bacterial implantation. Although the elevated blood pressure may be responsible also for the increased tendency toward

15. Nedzel, A. J.: Arch. Path. **24**:143, 1937.

myocarditis, it is likewise possible that the important factor is the temporary myocardial anoxemia resulting from the coronary spasm which Clark [16] showed to follow injection of pitressin. The anoxemia may in itself so lower myocardial resistance as to allow the virus to become established, or the momentary cardiac dilatation and drop in blood pressure concomitant with the closure of the coronary arteries may be significant.

The use of acacia was suggested by the work of Clark and Svec,[17] in which they were able to produce subacute bacterial endocarditis by preceding their infective dose of bacteria with a large dose of a solution of acacia, intravenously injected. These authors attributed the success of this method to the acute dilatation of the heart brought about by the sudden rapid increase in blood volume. It seems probable that the addition of this viscous, sticky material to the circulating fluids also brings about a slowing of the circulation through the heart and increased likelihood of the settling out of particulate matter. The demonstration of the mechanism involved awaits further experimentation.

The question as to whether virus III has some form of tropism for the heart or whether a variety of viruses act similarly under the same preparatory conditions is now under investigation. The fact that 25 per cent of a small series of otherwise "normal" rabbits showed evidence of cardiac damage attributable to this agent and that this has not been reported for any others suggests that there may be some specific affinity. The frequency with which lesions occurred in the adrenals is also interesting but unexplained.

SUMMARY

The intravenous, intratesticular or intranasal inoculation of virus III into rabbits resulted in the development of cardiac lesions in a small percentage of the animals. These lesions consisted primarily of scattered areas of myocarditis containing the characteristic intranuclear inclusion bodies in inflammatory cells, fibroblasts and muscle cells. The lesions occasionally included also nonvegetative endocarditis and fibrinous pericarditis.

If inoculation was preceded by puncture of the heart with a needle or by intravenous administration of a large dose of a solution of acacia the incidence of myocarditis approached 100 per cent, the severity and extent of the lesions were greatly increased, and endocarditis and pericarditis were frequent findings. Pitressin injected intravenously had a similar but less marked effect.

16. Clark, G. A.: J. Physiol. **68**:166, 1928.
17. Clark, P. F., and Svec, P. E.: J. Bact. **35**:55, 1938.

The importance of these observations is twofold. They demonstrate that a virus entering the animal body by several routes may localize in the heart and there cause extensive damage and that this localization presumably is largely dependent on some transient deviation from the normal physiologic conditions of the heart and circulatory system or on some minor and transient change in nutritional or anatomic relationships.

The possibility of a spontaneous virus III infection must be taken into account in the interpretation of experimental work dealing with cardiac disease in which the rabbit is used.

EFFECT OF POTASSIUM IODIDE ON BONE AND CARTILAGE IN THYROIDECTOMIZED IMMATURE GUINEA PIGS

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Potassium iodide injected intraperitoneally into immature male guinea pigs caused an increase in the proliferation and an acceleration of the differentiation of the euhyaline cartilage as well as resorptive processes in the osseous substance.[1] Since these changes bore a certain similarity to the lesions observed after oral administration of thyroid substance,[2] it seemed of interest to determine whether the effect of potassium iodide on cartilage and bone was direct, or whether it was brought about by the mediation of the thyroid gland, which in an early period is stimulated by this substance and in a subsequent period inhibited (Loeb[3]; Gray[4]; Rabinovitch[5]; McCordock[6]; Margolin[7]). For this purpose we studied the influence of potassium iodide on cartilage and bone in thyroidectomized guinea pigs.

MATERIAL AND METHODS

Eighteen male guinea pigs, weighing between 155 and 170 Gm. at the beginning of the experiment, were thyroidectomized. Of these guinea pigs, 6, which did not receive potassium iodide, served as controls. When the remaining 12 animals had reached the weight of 175 or 180 Gm., the administration of potassium iodide was begun. Six of these 12 guinea pigs received daily intraperitoneal injections of 0.01 Gm. of potassium iodide dissolved in 1 cc. of 0.9 per cent sodium chloride solution, and 6 others were given a daily dose of 0.05 Gm.

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These investigations were carried out with the aid of grants from the Committee on Scientific Research of the American Medical Association and from the International Cancer Research Foundation.

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of this substance, likewise by interperitoneal injection. Two animals, 1 from each of these two groups, were put to death after four, six, ten, twelve, fifteen and twenty days, respectively. At autopsy the region of the neck was searched for remnants of thyroid tissue, and if such remnants were found they were removed for microscopic examination. A tibia, a knee joint, ribs and vertebrae were removed for further study.

OBSERVATIONS

In 2 of the 12 guinea pigs which had been given injections of potassium iodide no thyroid remnants were found at autopsy or on microscopic examination. One of these animals had received four and the other twenty-one injections of 0.05 Gm. of potassium iodide. The thyroid tissue in the rest of the animals was hypertrophic and showed the changes that are known to follow administration of potassium iodide.[8]

Epiphysial Line of Upper Tibia.—After four injections of potassium iodide the epiphysial zone was of medium width and patent. In the intercartilaginous ground substance there was loosening associated with slight distention of the fibrillar network, which was filled with a light-staining fluid. The resting cartilage cells were found to be stimulated. The columnar cartilage cells proliferated by way of amitoses or, here and there, especially in the central portions of the epiphysial disk, by mitotic division. Simultaneously the nuclei enlarged and the cytoplasm became hypertrophic. Thus some of the ordinarily flattened columnar cells rounded off and resembled epithelioid cells. The hypertrophic cartilage cells had a regular structure and were sharply demarcated in a straight line from the bone marrow. No changes were observed as far as calcification, breakdown and replacement of the cartilage cells by bone were concerned. With increasing duration of the experiment the growth processes became more accentuated, and some retrogressive changes appeared also; both of these alterations reached a maximum about ten to fifteen days after the beginning of the injections. At that period, in the intercartilaginous ground substance fairly large wedgelike swollen areas were detected, where the fibrils were torn apart and the cartilaginous cell rows became irregular. Some cells had shrunk and disintegrated. Progressive changes went hand in hand with these retrogressive ones. The columnar cartilage proliferated freely and frequently by mitotic division; to a less extent also the resting cartilage cells increased in number. As was the case in the early stages of the experiment, no such increase in number was seen as far as the hypertrophic cartilage cells were concerned. The individual hypertrophic cartilage cells did not, however, seem to reach so large a size as did the corresponding cells in nonthyroidectomized guinea

8. Loeb, L.: Endocrinology **13**:49, 1929; Am. J. Path. **5**:79, 1929.

pigs. After twenty days the process of ossification was in progress, and a fairly large amount of bony substance had been deposited.

Chondrophyte.—In those instances in which proliferation was found in the epiphysial line, hyperplasia of the cartilage cells was likewise observed in the lateral protuberances. The conversion of precartilage into mature euhyaline cartilage cells was intensified and hypertrophic incubator capsules appeared. In the most advanced cases some of the hypertrophied cartilage cells underwent karyolysis and karyorrhexis. After twenty days a more resting condition was noticeable, although some hyperplasia was still present.

Joint.—The early changes consisted of narrowing of the layer of hypertrophic cartilage associated with accelerated corrosion by capillaries. After ten days the zone of the hypertrophic cartilage was to a large extent replaced by bone. In some areas the capillaries perforated the osseous lamella which ordinarily demarcates the epiphysial cavity from the cartilage. In addition, definite but moderate proliferation in the transitional and pressure zones was noted, which was somewhat more pronounced in those guinea pigs which had received the larger dose of potassium iodide. At about twelve to fifteen days after the beginning of treatment the cartilaginous covering of the joint appeared thickened, owing to the increase in the number of cells. The proliferating cells—four or more of which were packed together—showed a perpendicular arrangement and an increase in the size of the nuclei and cytoplasm. Some of these hypertrophied cartilage cells underwent degeneration and liquefaction. Similar changes were visible after twenty days; at this stage, however, ossification of the cartilage cells predominated markedly over the processes of hyperplasia and hypertrophy.

Bone Cavity.—The replacement of cartilage by bone took place in a normal manner at all stages. Strands of incompletely ossified or of nonossified cartilage, seen in corresponding animals with intact thyroid glands, were entirely missing. The trabeculae were thick and arranged in longitudinal and horizontal directions; they were surrounded by epithelioid cells, which multiplied mitotically. In the majority of instances these epithelioid cells were arranged in a beadlike manner; also, by fusion of several of these cells, giant cells were produced. In some places multinucleated cells accumulated and invaded and dissolved the bony substance. However, these resorptive processes were less pronounced than the appositional ones. In the early stages of the experiment the intercellular connective tissue was somewhat loosened, but after ten to fifteen days the fibers became denser, and, in particular, at the endosteal side of the bone slight fibrosis was found.

Bony Shaft.—The compact bone was thick. The haversian canals contained congested capillaries. In the periosteum the fibrillar network seemed slightly loosened at first and became denser and more sclerosed with increasing duration of the experiment. As in the case of the trabeculae of the bone marrow, so in the case of the bony shaft the apposition of bone apparently continued to progress, and there was no evidence of intensified destruction of osseous substance by either vascular canalization or cellular absorption.

Ribs and Vertebrae.—In ribs and vertebrae cartilage and bone behaved in very much the same way as in the long bones. The longer the time during which the potassium iodide was allowed to act, the more accentuated became both progressive and retrogressive changes of euhyaline cartilage. Ossification likewise was progressing. The periosteal tissue became increasingly sclerosed, and the combined changes which took place in cartilage, connective tissue and bone led to slight thickening of the chondro-osseous junction.

Effect of Presence of Remnants of Thyroid Tissue.—No significant differences could be established in the skeletal tissues of animals in which thyroid remnants were present and animals in which no thyroid tissue was found at autopsy or in subsequent microscopic examination. It appears, therefore, that small remnants of thyroid tissue were unable to alter appreciably such conditions in cartilage and bone as are observed after complete thyroidectomy.

COMMENT

Potassium iodide intensifies proliferation of the euhyaline cartilage in the completely or almost completely thyroidectomized immature male guinea pig. The degree of stimulation as indicated by the number of cartilage cells in the various cell rows of the epiphysial line and in the layers of the articular cartilage is about the same in the thyroidectomized animal and in the animal in which the thyroid is intact. However, the number of hypertrophic cartilage cells is normal in the animal in which the greater part of the thyroid gland has been removed, whereas it is increased in the guinea pig with an intact thyroid under otherwise similar conditions. More osseous substance is present and less absorption takes place in shafts and trabeculae, and ossification of the epiphysial lines proceeds in a more regular manner, in the thyroidectomized animal.

We might then assume that the increase in the proliferation of the euhyaline cartilage which occurs in both completely or almost completely thyroidectomized and nonthyroidectomized guinea pigs after administration of potassium iodide does not depend on the presence of the thyroid gland.

While this would hold good in a general way, there are indications that, after all, the thyroid hormone may play a certain limited role in this process. The lesser tendency of the cartilage to undergo hypertrophy in the thyroidectomized animals supports the view which we[2] have previously expressed, namely, that the thyroid hormone in this stage affects the endochondral osteogenesis. The increased number of hypertrophic cartilage cells seen in guinea pigs with intact thyroid glands under the influence of potasium iodide would then be a secondary effect, due to stimulation of the thyroid gland.

The deposition of larger amounts of osseous substance in completely or almost completely thyroidectomized guinea pigs indicates a decrease in absorptive activity and represents a reversal of conditions which are observed in hyperthyroidism. Thus the increase in absorption of bone and the simultaneous decrease in deposition of new bone which occurred in animals with intact thyroid glands under the influence of potassium iodide may also have been due to the mediation of the stimulated thyroid gland. The possible influence of thyroidectomy on growth and ossification of cartilage will be discussed at a later date.

SUMMARY

In immature male guinea pigs in which the thyroid glands were completely or almost completely removed potassium iodide caused proliferation of the euhyaline cartilage as it does in nonthyroidectomized animals. In this respect potassium iodide acts on the growth of the cartilage without the mediation of the thyroid gland. However, the increase in the number of hypertrophic cartilage cells, the decrease of ossification and the stimulation of resorption of bone which occurred when the thyroid gland was intact were lacking when the thyroid gland had been removed previous to the administration of potassium iodide. These processes, which under the influence of potassium iodide are seen only in guinea pigs with intact thyroid glands, are held to depend on certain functions of the thyroid gland.

ROLE PLAYED BY TRAUMA IN THE DISSEMINATION OF TUMOR FRAGMENTS BY THE CIRCULATION

TUMOR STUDIED: BROWN-PEARCE RABBIT EPITHELIOMA

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The mechanism by which metastases develop in the host of a malignant transplantable tumor is of considerable interest.[1] Trauma to the parent tumor followed by dissemination of fragments by means of the blood stream is an obvious explanation of one way in which tumor cells or masses may be spread to distant organs. The purpose of this paper is to describe a rather common and simple mechanism for the dissemination of malignant cells as a result of trauma which we have observed in the course of our experiments with the growth of tumor blood vessels in the ear chamber.

In previous papers in this series [2] the method of introducing a transparent window into the rabbit's ear and planting tumor beneath it was described, together with the sequence of events in the development of the vascular supply to the growing tumor and the characteristic pattern of growth of large, irregular, thin-walled, bulbous vessels in the growing tumor edge. The growing tumor margin is noted in the chamber as a fine, rather opalescent filamentous and feathery edge around which and into which are seen to grow a large number of new blood vessels. A

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2. (*a*) Downing, V.; Warren, S. L., and Bishop, F. W.: Effects of Roentgen Irradiations upon the Blood Vessels of Repair Tissue and the Brown-Pearce Rabbit Epithelioma, Am. J. Roentgenol., to be published. (*b*) Ide, A. G.; Baker, N. H., and Warren, S. L.: Vascularization of the Brown-Pearce Rabbit Epithelioma Transplant as Seen in the Transparent Ear Chamber, ibid., to be published.

great many of these channels are large enough to support 10 to 20 red blood cells abreast. A few are even ten to twenty times this size. These are connected by frequent anastomoses. They are simple endothelial tubes with little or no supporting structure in their walls, the tumor cells being adjacent to the endothelium. Following a slight trauma, the edge of the tumor and the repair tissues around the tumor become obscured by the outflow of a large number of red corpuscles without clotting. This completely obscures all the vessels in the area. Within a few hours a great number of red blood cells have disappeared from this field, allowing the larger blood vessels to stand out rather prominently, although the smaller connecting vessels are still obscured. A definite current with backward and forward flow of red blood corpuscles may now be seen extending through this area of hemorrhage and frequently along the margin of the growing tumor. Among these red blood cells eddying backward and forward across the filamentous, feathery edge of the growing tumor margin are occasionally seen small, irregular, rather opalescent masses of tissue resembling in every way small fragments of tumor cells which have apparently broken loose from the growing tumor margin and now lie free in the fluid red blood cell mixture. Several of these have been seen to float with the current of red blood cells out of the field of vision in the direction of some of the larger vessels. In general the current in the extravasated blood flowed from the growing tumor margin, presumably originating from vessels within the tumor or below the tumor, across the field into large vessels (apparently veins) in the surrounding recently repaired tissue where the ruptured vessels were apparently open to receive it. After the regrowth of the endothelium and repair of the ruptured vessels, the opalescent fragments were no longer seen.

It may be said that this situation, namely, hemorrhage along the growing edge of the tumor in the chamber, is distinctly different from that which might arise in a spontaneous malignant tumor or in a transplanted tumor mass growing in the testes or elsewhere in the body. An illustration of how this is possible within the tumor mass was visualized in colored moving pictures taken of some of the blood vessel structures within a growing tumor mass. By good fortune, a portion of the tumor was chosen in which a minor apparently spontaneous trauma had occurred, resulting in a split through the tumor (between *5* and *6* in fig. 1) and across three vessels (*2, 3* and *4* in fig. 1) with separation of the latter from their connection with a large vessel (*2* in fig. 1). In the lower corner of the field, shown diagrammatically in figure 1, was a large vessel (*1* in fig. 1), presumably a venule by its dark color, the diameter of which was equal to several hundred red blood corpuscles. The convex surface of this vessel at the curve (bend) had apparently

lost most of its endothelial wall, since the stream of red blood cells at this point eddied out beyond the contour of the nearby walls and no continuity of wall could be seen at its margin. The endothelial wall had been ruptured or had been stripped off for a distance equal to the diameter of several thousand red blood cells. Since the torn portion was

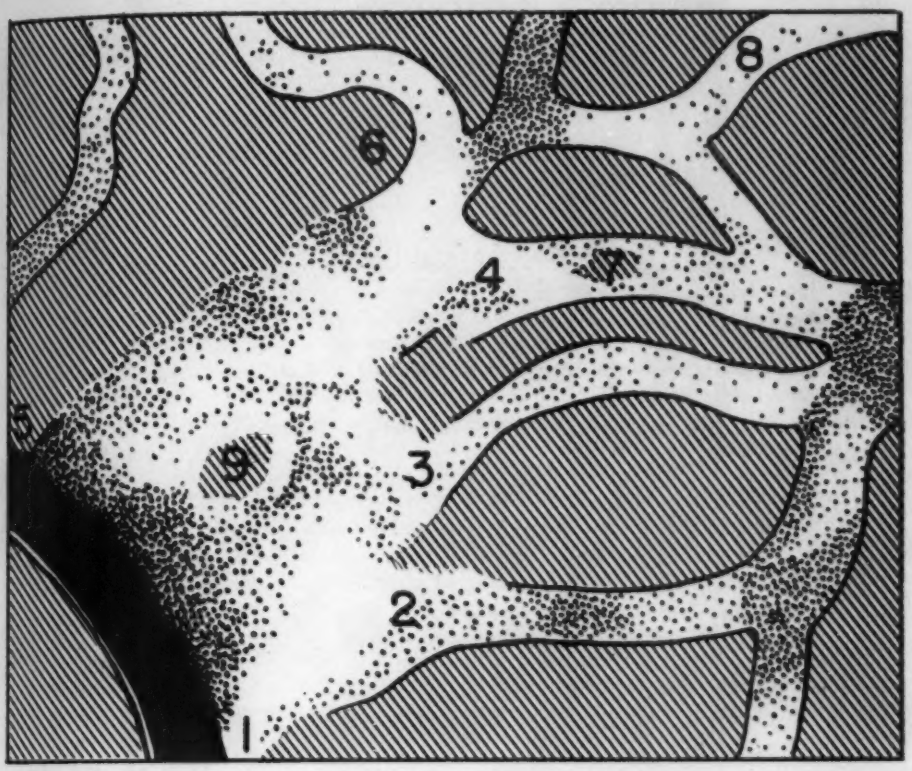

Fig. 1.—A composite schematic view (from the colored moving pictures) of a tear from vessel *5* to vessel *6* and across the vessels *2, 3* and *4* through a portion of actively growing Brown-Pearce rabbit epithelioma, near the growing edge. A portion of the tumor has been shifted along the torn edge of a venule, *1*, from *5* to *2* leaving an island of tumor tissue at *9*. The border from *5* to *6* and the peninsulas between vessels *2, 3* and *4* are made up of ragged, friable tumor cells. A loose fragment of tumor cells, *7*, is shown in vessel *4* at one stage of its journey from near vessel *5*, back and forth through vessels *4* and *8*, and then out of the field by way of *8* as described in the text. Red blood cells in loose masses and widely separated groups wash back and forth in the plasma (clear zones). No evidence of clot or fibrin formation was noted in this or other similar traumatized areas of this size. The current of the venule, *1*, was uninterrupted; red blood cells drifted in and out of the periphery of the stream in clouds and whirls through the widely torn aperture on the convexity of the venule. It is easy to see how a mass of tumor cells, *7*, could be swept into this venule through such a tear or how it might pass from the vessels *4* and *8* into other venules nearby.

EXPLANATION OF FIGURE 2

Individual frames from a 16 mm. kodachrome, taken at 4 frames per second, enlarged approximately forty times. Vessels show only when full or partly filled with red blood cells. The dimly seen vessels are out of the focal plane.

The upper frame shows the whirls of red blood cells at the torn convex margin of the venule (near *1* in fig. 1). The current in the venule continued (upward and to the left) without interruption in spite of the defect in its wall. At the upper left corner may be seen a ragged cloud or loose mass of red blood cells, which swirl around in the plasma. The clear zone in the center is an island of tumor (*9*). Vessels *2, 3* and *4* of figure 1 are out of the field to the right.

The middle frame is confusing because of the multiplicity of vessels at various levels. The tumor fragment (*7* in fig. 1) in the circle is in vessel *4* in figure 1 near its junction with vessels *3* and *8* (not visualized here because they contain no red blood cells at the moment).

The lower frame shows the fragment (*7* in fig. 1) near a space free of red blood cells just before it entered vessel *8* in figure 1, which is not seen here because it is filled with plasma only. The color and the projection give greater clarity in the identification of details.

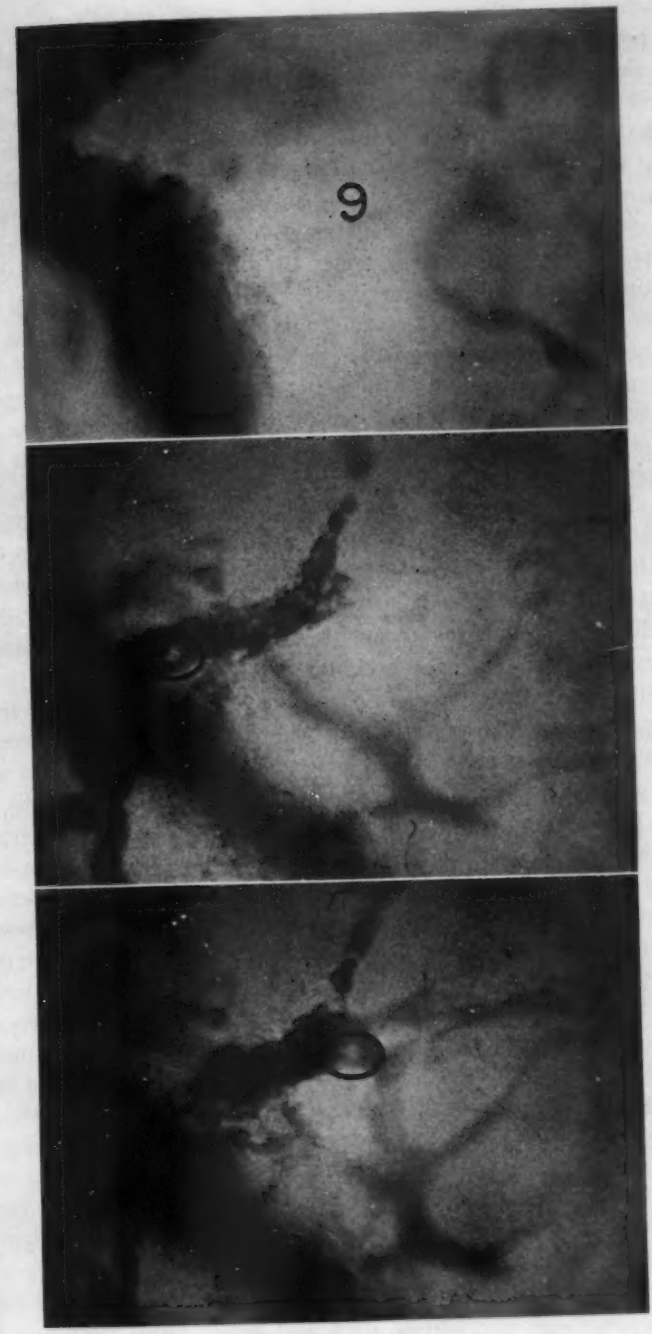

Figure 2

well within the tumor mass a closed system was still operative, so that it was possible for the main flow of blood to continue its original direction in the venule. Small losses of cells and plasma from the periphery occurred now and then as the connecting pressures in the nearby and connected vessels varied from moment to moment. This produced the washing to and fro previously described. In the upper and right hand portion of this area were seen the aperture and straight margins of several communicating vessel walls, the remainder of these vessels having been ripped apart (*2, 3* and *4* in fig. 1). In between these small communicating vessels and the larger vessel (vein) were several small islands of tumor tissue which had remained intact and in position (*9* in fig. 1; fig. 2*A*). At the upper margins of the area were several other ill defined masses apparently of tumor tissue between the sites of several capillaries or smaller vessels. Red blood cells were passing through the larger venule at a fairly high rate of speed, and a few were eddying off of its periphery into the spaces between the masses of tumor tissue. These red blood cells were mixed rather sparsely with plasma or diluted with tissue juices or plasma. Either single red blood cells or masses of red blood cells were whirled around here and there in between the masses of tumor cells and finally back into the periphery of the rapidly flowing blood cells in the venule together with the red blood cells which streamed down from, or washed back and forth in the upper part of the area presumably from other vessels. The field was then moved upward and to the right two fields to a position where the intact, large, irregular, so-called capillaries were visible. The current of blood in these vessels was in general downward and to the left apparently into the large venule which had the rupture in its walls. This was verified by repeated observations before and after the moving picture was made. The flow of blood in these large capillaries was quite erratic, having in general a to and fro movement or a washing back and forth of flow with, however, the general current in the direction of the large venule. Large mobile masses of red cells interspersed with clear plasma and small groups of red blood cells could be seen at different times. No clotting was noted at any time on the exposed tumor surfaces or within the fluid medium. At one interval, a small, irregularly shaped, opalescent mass (*7* in fig. 1), approximately 5 to 10 red cells in diameter and 40 to 50 red cells long, was seen to come from the torn margin of the tumor in the region of *5* in figure 1. It moved upward and to the right across and out of the field by way of vessels *4* and *8*. It is illustrated in vessel *4* (fig. 1) during its change of position. This bit of fragment was evidently tumor tissue and not fat, clot or some other extraneous material, for it was opalescent like the other tumor cell masses and kept its general volume and irregular shape on turning over and being rolled back and forth

by the blood current. While the film was being made and later, several similar small opalescent fragments of different shapes were seen to drift down into the general region of the venule and disappear.

COMMENT

It can be readily seen that with a trauma of this type a great many loose fragments of torn tumor tissue, dead or alive, could be carried into the general circulation in this manner. These small fragments mix with masses of red cells and plasma without the formation of a clot. Since the volume of flow of the plasma and blood cells is quite large in these large "capillaries," fragments could easily be carried down into the venous system and thus into the general circulation. The area described was not observed long enough to determine whether repair with sealing off of the vessels occurred in this region, but from previous experience with this type of tissue we may assume that in a very short time the margins of the flowing current of the cells and plasma were lined with endothelium and a barrier erected against further dissemination of tumor fragments from this location.

From the previous paper,[2b] the development of large-sized, so-called capillaries in a haphazard, abandoned manner may be seen to occur with great regularity at the active growing tumor margin. In general these capillaries have a wall which consists almost entirely of thin endothelial cells with practically no visible supporting stroma. Such a vessel is apparently surrounded by actively growing tumor tissue whose cells are in close apposition to the vessel walls. These vessel walls are very friable and easily broken. The observation that red blood corpuscles and plasma may break loose into the tumor (to be reported later) without the development of fibrin or clot is confirmed by observations on other tissues, particularly the mesentery, where collections of several thousand red blood cells may extravasate from the torn vessel channel without the occurrence of clotting.

It is not uncommon to see in tumor tissue a microscopically massive or even grossly visible hemorrhage without clotting. The general mass of red blood cells and plasma still communicates with several open blood vessel lumens. A current soon develops which will flow across between the most direct connections and set up in among the broken and split growing tumor tissue massive channels of blood flow. Apparently endothelium grows out from every open channel and every remnant of a capillary wall and tends to grow along the margin of the current of the flowing blood and plasma. When these connections all meet, an intact wall is produced, forming large and small sinusoids of bizarre shape. In the meantime, loose tumor fragments may be wafted down the current of the plasma and cells and are thus able to get into the

gaping openings of large capillaries or venules. Since many of the so-called capillary vessels in the growing tumor margin are 50 red cells in diameter or greater, one may readily see how relatively large fragments may be borne from the original tumor site to the lungs or other organs.

Within the experience of this laboratory, metastases have not developed in any of the organs in the rabbit when the tumor was transplanted into the ear either subcutaneously or under a window (105 cases). When the tumor is transplanted in the ear by whatever method, its growth tends to be self limited by the physical and anatomic restrictions placed about its expansion. It soon regresses and dies out, its place being taken by a repair reaction. When transplanted subcutaneously, it grows to a large nodule, 2 or 3 cm. in diameter, which soon ulcerates and frequently sloughs out and becomes infected; the growing tumor disappears and a scar is produced. A high percentage of these animals are refractory to subsequent reinoculations.[3] On the other hand, intratesticular transplantation is followed by rapid development of a mass in susceptible stock, with a high percentage of generalized metastases. As with human tumors, the size and appearance of the metastatic nodules are such as to suggest separate showers with dissemination from the primary tumor or even from the older (secondary) metastatic nodules.

From the episodes described in this paper it is evident that many tumor fragments may break loose from the site in the ear and get into the general circulation. It is interesting to speculate on the fact that they do not in such a case give rise to metastases elsewhere.[4] It is probable that they are stopped in the main by the pulmonary capillaries. It has been the experience of other workers besides those in this laboratory that this tumor will grow readily if it is well ground up, and a suspension injected intravenously into a susceptible rabbit. In this laboratory unreported experiments by Jares and Sahler and ourselves have demonstrated that tumor inoculated into the ear vein or the jugular vein in this manner will show many takes in the body other than in the lung. The lungs are studded with a fine tubercle-like growth of tumor which is rather diffuse in distribution but which has not formed any large nodules or aggregate tumor masses. On the other hand, there are, at the same time, aggregates or masses in the liver, kidneys, ovaries and other organs. Since these animals were not put to death until late, it cannot be determined with certainty whether these distant tumor nodules originated from the initial inoculation or whether they were secondary transplants from tumor which was thrown out from the growing tumor in the lung.

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In rabbits showing metastatic nodules from testicular implants the lungs are frequently involved, yet one has the impression that the involvement of the liver, heart, mediastinal structures, kidneys and other tissues is more widespread and the growth more vigorous. This suggests that it is perhaps more difficult for the tumor to get a foothold and to grow as actively in the lungs as elsewhere. Whether this is dependent on the metabolic activity or the mechanical activity of this organ is difficult to say. Certainly from the standpoint of filtration alone, if our observations are correct, the lungs (and the liver) are strategically placed to be overwhelmed by the numerous fragments disseminated from the tumor by trauma. That this must occur frequently is obvious. The study of the defense mechanism against the growth of the disseminated fragments while difficult should be of great interest.[5]

From observations of colored moving pictures of the mesentery capillaries, previously unreported, it is obvious that fairly large globules of what is apparently fat in the mesentery, capillaries and venules may pass through the capillaries with great ease. A good many of these are from 10 to 50 or 60 red blood corpuscles in length and completely fill the capillary. They seem to have no difficulty in following the blood flow down the capillary channel to the venule, where they become globular as the larger diameter of the vessel is able to accommodate them. It is highly probable that in the capillaries of the lungs fairly large-sized particles of tumor tissue, especially if they are roughly cylindric, might well pass through the capillary bed of the lung tissue. If the inoculum is a filtered emulsion, in which the size of the tumor cell masses is relatively small, or if a trauma has occurred such as has been described, it is probably, if not actually, quite frequently the case that masses of tumor tissue in suitable shapes may well pass into the venous circulation and then through the capillary bed of the lung tissue and so get into the general circulation. It is then a matter of chance where one of these may lodge in a distant structure and whether, after lodging in the capillary bed of a distant organ, it is capable of establishing growth there or is destroyed or phagocytosed.[5] It is of course not clear why metastases are able to grow in distant organs or, on the other hand, why they grow at all in sites distant from the origin. Is it the size of the disseminated cell mass or the number of masses lodged in a given vascular site which determines the local take or is it related entirely to the viability of the cells? Having been caught in a vessel, the tumor fragment in its growth need not depend on autolysis of the endothelium by anything elaborated by the tumor. Certainly from Clark's[6] obser-

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vations in capillaries which may show no blood flow for a while, the endothelium breaks and retracts or pinches off. Such a mechanism is simple and would leave the tumor cells free in the extracellular juices of the tissue. Growth or death of the tumor tissue from this point on is determined by factors beyond our knowledge at present.

SUMMARY

Blood vessels developing within the Brown-Pearce rabbit epithelioma transplanted into an ear chamber are extremely friable, since even large vessels (50 to 200 red blood cells in diameter) may have walls consisting only of endothelium. Small tumor fragments that have broken loose near a ruptured vessel may readily be swept into the large capillaries and thus into the general circulation. This seems to be a rather common occurrence in the tumor growing in the ear chamber, yet the development of tumor metastases has not been noted in over 105 such experiments. Study of the local defense of body organs, particularly the lungs, against tumor fragments should be of considerable interest.

Case Reports

MULTIPLE MENINGIOMA WITH SARCOMATOUS TRANSITION IN ONE NODULE

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A summary of the literature on multiple meningioma was made in 1935 by Raaf and Craig,[1] who included the earlier list of Hosoi [2] (1930) and added 6 recent cases. They also reported in full a case in which they successfully removed three meningiomas at operation.

Further search of the literature reveals other reports as follows: Penfield and Young [3] recorded the case of a 21 year old woman in whom postmortem examination showed, in addition to multiple meningioma, multiple neurofibromatosis of the cranial and peripheral nerves, an ependymoma of the cord and cellular astrocytomas of the cord, cerebrum and cerebellum. Perrero and Pitotti [4] recorded the finding of one cerebral and three spinal meningiomas in a man aged 31. Alajouanine and co-workers [5] reported numerous meningiomas in a 52 year old woman. These were associated with bilateral acoustic neuromas and multiple fibromatosis. Woltman and Love [6] operatively removed two meningiomas from a 43 year old woman. Harbitz [7] recorded the case of a 5½ year old girl who showed, in addition to meningiomatous nodules, a diffuse invasion of the meninges by similar tissue. He stated that the tumor cells in some areas resembled sarcomatous cells, but apparently the evidence was not sufficiently definite to warrant a diagnosis on this basis. He also mentioned 2 previously unreported cases: the patients were a man aged 55 years, the number of tumors in whom was not stated, and a 72 year old woman, in whom two meningiomas were found. Arlt [8] reported a case which was associated with diffuse meningiomatosis of the spinal cord. Worster and associates [9] described 2 cases and Pacifico [10] 1 case, the patient in the latter case presenting

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6. Woltman, H. W., and Love, J. G.: Proc. Staff Meet., Mayo Clin. **10**:497, 1935.
7. Harbitz, H. F.: Acta path. et microbiol. Scandinav. **12**:24, 1935.
8. Arlt, H. G.: Ztschr. f. d. ges. Neurol. u. Psychiat. **156**:713, 1936.
9. Worster, D.; Dickson, W. E. C., and McMenemey, W. H.: Brain **60**:85, 1937.
10. Pacifico, A.: Riv. di pat. nerv. **50**:299, 1937.

also internal hemorrhagic pachymeningitis. Horrax [11] mentioned 4 patients in whom multiple meningiomas were found.

This brings the number of reported cases up to 44. In none of these was evidence of malignant change found, although as stated, Harbitz [7] mentioned that some of the cells in his case resembled those seen in sarcoma. It was therefore considered worth while to report this case in which one of a number of leptomeningiomas (classification of Globus [12]) was found to be highly malignant.

REPORT OF A CASE

A 32 year old man was admitted to the Pierce County Hospital, Tacoma, Wash., Aug. 13, 1938, with the complaint of severe attacks of headache. A report from his local physician stated that prior to hospitalization he was found to have bilateral papilledema and exaggerated reflexes, leading to a diagnosis of tumor of the brain.

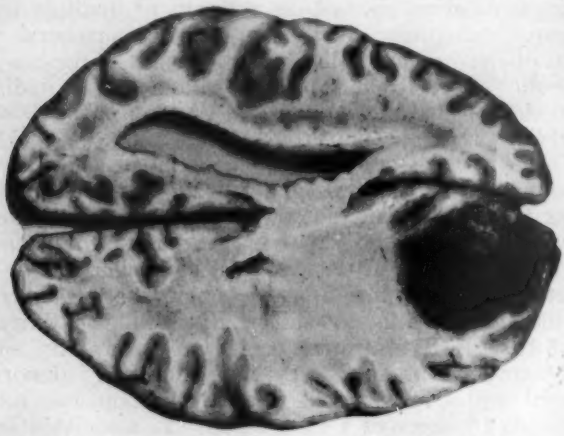

Fig. 1.—Photograph showing the largest meningioma.

After entry into the hospital his headaches were accompanied by nausea and vomiting. A spinal tap revealed a pressure of 58 mm. of mercury. The following day he became noisy and irrational, and on the morning of the third day after admission he began to froth at the mouth, became cyanotic and died within thirty minutes.

Autopsy.—Postmortem examination of the brain revealed the dura to be intensely hyperemic, the subarachnoid space dry and the pial vessels dilated. There was marked flattening of the cerebral convolutions, and the brain was asymmetric, the anterior portion of the right hemisphere appearing larger than that of the left. The vessels at the base were well preserved and of normal distribution but were rather small and hypoplastic. The cerebellar tonsils were elongated, indicating compression from above. The cerebrospinal fluid was clear.

On the outer surface of the dura were 20 to 30 small nodules, the largest measuring about 0.5 cm. in diameter. These were present in greatest number on

11. Horrax, G.: Arch. Neurol. & Psychiat. **41**:140, 1939.
12. Globus, J. H.: Arch. Neurol. & Psychiat. **38**:667, 1937.

the dura lining the floor of the left anterior and middle fossae. Projecting through the bone in the central portion of the left anterior fossa was a nodule about 3 mm. in diameter. The bony floor of both anterior fossae was very thin and could be cut with scissors. On the surface of the dura overlying the superior

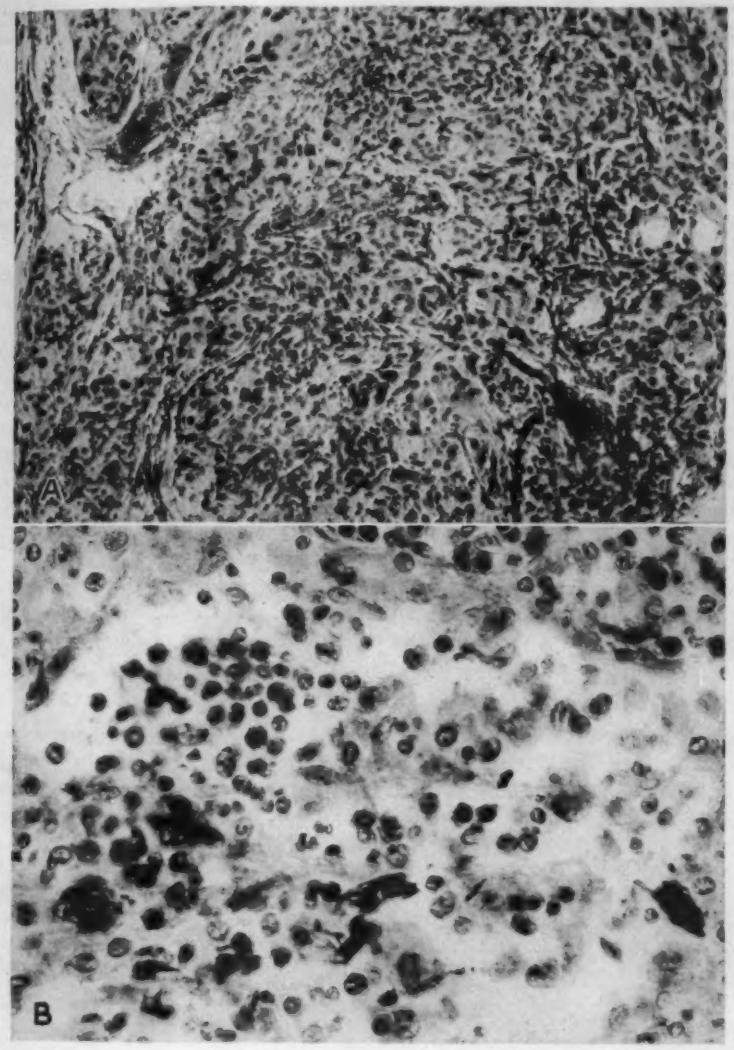

Fig. 2.—*A*, aborted whorl formations; *B*, sarcomatous transition of cells.

portion of the right frontal lobe was a large, firm, pale reddish gray tumor mass, which had extended through the dura and produced adhesions of the dura to the calvarium, with erosion of the overlying bone. About 3 cm. posterior to this was a small nodule, lying in the midline.

On the cut surface of the brain, after fixation in solution of formaldehyde U. S. P., the large mass just described was found to have invaded the right frontal lobe to a depth of several centimeters, replacing to a considerable extent the white matter of the centrum ovale. The gray matter on the superior and mesial surfaces was completely destroyed. The widest diameter of the tumor measured 6 cm. The irregular mass was studded throughout with small foci of gelatinous degeneration, and posterior to the main body of the tumor was a larger area of similar degeneration protruding into and grossly distorting the anterior horn of the lateral ventricle. The septum pellucidum had been pushed to the opposite side, partially obliterating the left foramen of Monro, and all portions of the left lateral ventricle were considerably dilated. The left frontal lobe was much smaller than the right, and the same was true to a lesser extent of the other portions of the left hemisphere (fig. 1).

Microscopic Examination.—The large invasive tumor mass was composed chiefly of endothelial cells which showed a distinct tendency to whorl formation (fig. 2 *A*). It was well vascularized and had a scanty connective tissue stroma. The tumor as a whole was cellular, the individual cells varying in appearance, depending on the portion of tissue examined. The most differentiated cells were found in the areas of whorl formation, where they were closely packed together. Each had a medium-sized nucleus and a spindle-shaped body with scanty, clear cytoplasm. In the regions where the whorl formation was not so marked, the cells had a much more embryonic appearance. Here there were giant cells, large mesenchymal cells and numerous cells in which all stages of mitosis might be observed (fig. 2 *B*). Sections of the adjacent brain tissue showed small plugs of embryonic tumor cells infiltrating the parenchyma. In addition to the larger areas of gelatinous degeneration described in the gross specimen, numerous microscopic areas of this type were present.

The smaller nodules were composed of adult-appearing endothelial cells, showing a more distinct tendency to whorl formation. The component cells of these tumors did not present the embryonic appearance and lack of differentiation seen in the tumor previously described.

SUMMARY

A case of multiple leptomeningioma is reported, the unusual feature of which was the sarcomatous change in the largest of the growths. This growth had invaded both the calvarium and the underlying brain parenchyma, with gross destruction of the entire right frontal lobe. There was a marked increase in intracranial pressure.

On review of the literature many instances of multiple meningioma were found described, but in only one description was mention made of the presence of cells resembling sarcoma cells. A single tumor somewhat similar to the one described in this paper was reported by Globus [12] and classified as leptomeningioma with a tendency toward sarcomatous transition.

INTRACAPILLARY MICROSCOPIC METASTATIC MAMMARY GLAND CARCINOMA OF THE LUNGS AND OTHER VISCERA

LESLIE R. GRAMS, M.D., CHICAGO

The lungs are invaded secondarily by carcinoma through the lymph channels and blood vessels. With vascular extension, tumor tissue emboli are carried into small divisions of the pulmonary artery and into the capillaries. Here they either are destroyed or by continued growth become implanted in the lung tissues. Many, but not all, pulmonary metastases have sufficient size and contrast, or both, to be found by gross examination. Schmidt [1] emphasized that microscopic examination would disclose metastatic carcinoma in lung tissues when no tumor had been detected grossly. He demonstrated microscopic tumor emboli in pulmonary arterioles in 15 of 45 cases of abdominal carcinoma. The tumor tissues in 5 cases were limited to intravascular thrombi. Schmidt believed the transport of the tumor cells into the lungs was through the lymphatics and thoracic duct to the venous system. Hemic transportation following carcinoma invasion of the blood vessels in the region of the primary growth is another possibility.

According to experiments in rats by Warren and Gates,[2] carcinoma cells when injected intravenously lodged in pulmonary capillaries, usually at a bifurcation, the site being determined by mechanical factors. The implanted cells grew from the capillaries to the alveoli by the third or fourth day. The establishment of these metastases was determined by the initial vitality of the cells and their prompt extravascular growth, because the tumor cells did not survive in the blood stream.

The factors which influence the distribution and the ability of the transplanted tumor tissues to grow in remote organs are not understood. There are several hypothetic considerations. Some observers have emphasized mechanical and quantitative factors and suggested that the character of distribution and growth is determined by the extent of vascular invasion. Virchow [3] stressed the constitution and metabolism of tissue. He observed that metastases usually are absent where primary carcinomas are frequent and that secondary growths occur where primary growths are rare. Ehrlich [3] suggested an "athreptic immunity"; i. e., certain organs have specific substances that promote growth, which others lack. Metastatic implantation has been explained on the basis of local

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John Jay Borland Fellow.

From the Henry Baird Favill Laboratory of St. Luke's Hospital.

1. Schmidt, M. B.: Die Verbreitungswege der Karzinome und die Beziehung generalisierter Sarkome zu den leukämischen Neubildungen, Jena, Gustav Fischer, 1903.

2. Warren, S., and Gates, O.: Am. J. Cancer **27**:485, 1936.

3. Cited by Oertel, H.: J. Path. & Bact. **40**:323, 1935.

changes in the physiologic activity and metabolism of tissue. Cohnheim [3] believed that a loss of physiologic resistance enabled the tumor cells to adhere to and replace normal tissue. When tumor cells are transported, the invaded tissues respond by supplying stroma, vessels and nerves. The transported cells thereby can develop into a tumor. It appears, accordingly, that an important factor in metastatic implantation is the reaction of the host tissues to the implanted tumor cells.

Ceelen [4] described an extensive intravascular growth of carcinoma and stroma in the lungs of a white man aged 27 years, secondary to a gastric tumor. Multiple gray nodules, 1 to 2 mm. in diameter, were scattered in the lungs. The right side of the heart was hypertrophied and dilated. Histologic examinations disclosed extensive thrombosis of the small divisions of the pulmonary artery. The thrombi were recent and organized. The freshly formed thrombi contained nests of tumor cells; those organized did not. Ceelen believed that emboli of tumor cells lodged in the pulmonary vessels and there stimulated an inflammatory response.

The conditions when metastatic carcinoma cells grow in the capillaries of the lungs without producing inflammation are distinct modifications. They simulate the relations in parasitic infestations, in which the host supplies merely a habitat medium and nutriment for the parasite, without a tissue response. Such an unusual intravascular growth of metastatic tumor in the capillaries of the lungs was observed with a primary carcinoma of the mammary gland.

REPORT OF A CASE

A white woman aged 44 was admitted to St. Luke's Hospital Feb. 7, 1938, in the care of Dr. George V. LeRoy. She had been treated elsewhere and was sent to this hospital with a diagnosis of generalized metastatic carcinoma and secondary anemia. The erythrocytes were 2,790,000 and the leukocytes 46,000 per cubic millimeter; the hemoglobin was 8.5 Gm. per hundred cubic centimeters. Her condition did not appear serious. Twenty-two hours later she suddenly became pale and pulseless, had gasping respirations and died.

In 1929 she underwent a thyroidectomy from which she recovered promptly. In 1936 a small hard mass was noted in the upper right clavicular region, another, about 3 cm. in diameter, in the right axillary region, and a third, about 3 cm. in diameter, in the upper medial portion of the right breast, above the nipple. The mass in the breast was considered carcinoma; the others, axillary and supraclavicular metastases. Preoperative roentgen therapy over the right breast amounted to 4,240 roentgens (r) over three portals, 1,665 r over the right breast, 2,015 r over the gland of the cervical region and 560 r over the right axillary region. Right mastectomy was performed, and the patient had an uneventful recovery. She complained of severe pain over the sacrum and in the legs. Roentgen therapy, to the extent of 3,000 r, was given in this region. The blood was unchanged until shortly after the second series of roentgen treatments, when the hemoglobin dropped progressively to 8 Gm. and the erythrocytes to 2,680,000. She received one transfusion and iron medication. Her condition improved for one year, then the hemoglobin fell to 8.9 Gm. and the erythrocytes to 2,170,000 per cubic millimeter.

4. Ceelen, W.: Med. Klin. **16**:95, 1920.

The essentials of the postmortem examination (Edwin F. Hirsch) are as follows. The right side of the chest bore a surgical mammectomy scar. The right axillary fossa presented considerable scar tissue. The abdomen contained about 500 cc. of a clear yellow fluid. The peritoneum was smooth, moist and glistening. There were no unusual changes in the pelvis. The lower border of the left lobe of the liver was 14 cm. below the xiphoid process in the midline. There were fibrous adhesions between both lungs and the wall of the chest. The right side of the chest contained about 100 cc. of limpid fluid. The heart weighed 380 Gm. The thickness of the myocardium of the right ventricle varied from 5 to 7 mm.; that of the myocardium of the left ventricle was 1.4 cm. All chambers of the heart were dilated. There was chronic passive hyperemia of the liver, spleen and other viscera.

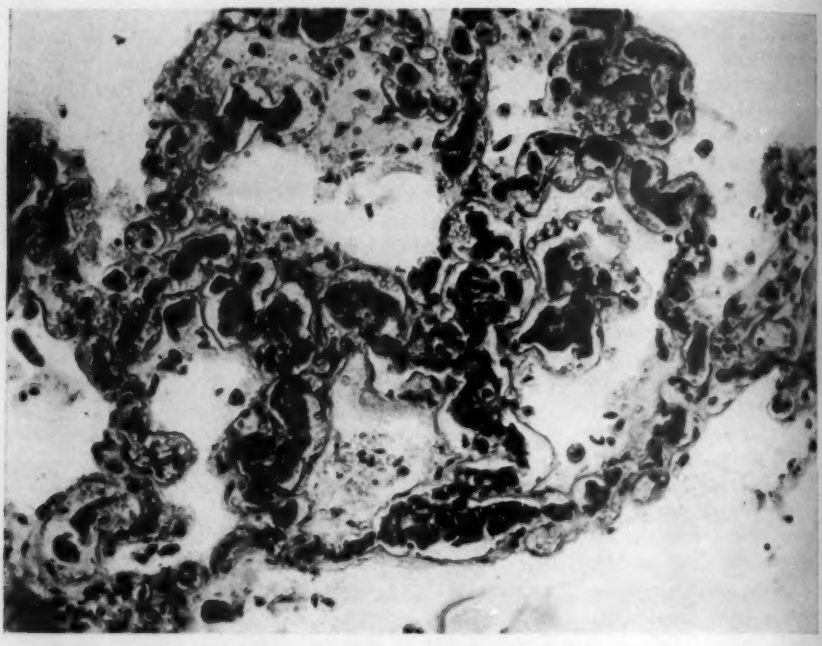
The right lung weighed 455 Gm. The visceral pleura was roughened by fibrous adhesions and mottled with carbon. The lung tissues were leathery and indurated: there were no nodules. On surfaces made by cutting, the tissues were gray and contained a frothy fluid, except the posterior portion, which was red-brown. The lining of the bronchi, traced out to the smaller divisions, was slightly hyperemic but smooth. There were no unusual changes of the pulmonary arteries or veins, opened to their smaller divisions. After inflation and fixation in 10 per cent formaldehyde solution the surfaces made by cutting showed dark gray indurated tissues. The left lung weighed 410 Gm. There were a few fibrous adhesions at the apex; otherwise the left lung resembled the right.

Many of the alveoli of the lungs contained mononuclear phagocytes with black pigment granules and desquamated lining cells. The smaller arterioles contained masses of carcinoma cells and red blood cells. The lumens of numerous capillaries (figure) in the alveolar walls had small cords and masses of tumor cells molded to the channels. Many tumor cells were in mitosis. The alveolar walls were thickened slightly by fibrous tissue. The changes were most marked in the centers of the primary lobules about the arterioles. Toward the periphery the alveolar walls approached the usual thickness. The tumor cells did not penetrate the capillary walls and replace the alveoli. Some of the larger arterioles also contained carcinoma cell aggregates, but the proportion of the lumen occupied was considerably less than that occupied in the smaller arterioles. The pleura showed no unusual changes. Both lungs were alike.

There were no unusual changes of the aorta, gallbladder, kidneys, fallopian tubes, uterus, lymph nodes, diaphragm, brain, stomach or small and large bowel. A few small vascular spaces in the submucosa of the appendix vermiformis contained masses of tumor cells without tendency to invade the surrounding tissues. There were similar masses in some of the myocardial arterioles and capillaries. The sinusoids of the spleen had small masses of carcinoma cells without perivascular invasion. The invasion of the small vascular channels in the adrenal glands was considerable. Some cells had penetrated the vascular walls and invaded the parenchyma. The involvement of the ovaries was similar to that of the tissues of the adrenal glands.

The lungs from 5 patients with primary carcinoma of the mammary gland and pulmonary metastases were compared. Secondary growths were found in all the lungs by gross examination. In the histologic preparations the carcinoma cells were in the alveoli and perivascular and peribronchial lymphatics. Blood vessels were involved occasionally, but the growth of tumor tissues was not restricted to the capillaries. The portions between the tumor masses were unchanged.

The possibility of a relationship between the extensive roentgen therapy and this unusual intravascular growth of carcinoma deserves some consideration. Hunter [5] reported a carcinoma of the mammary gland with generalized spread following radium treatment. There are a few other reports of similar observations. Radiation therapy, however, is not considered to be a factor in the dissemination of carcinoma; it prolongs life and thereby increases the opportunity for metastases to develop. Ciro and Bolestra [6] observed that roentgen irradiation of mice with tar carcinoma markedly increased the incidence of metastases. They concluded that radiation decreased the resistance of the tissues and rendered tumor growth possible.



Photomicrograph illustrating the intracapillary masses of metastatic carcinoma of the lung; × 198. Those in other viscera were similar. Note the lack of tissue reaction about the tumor cells.

SUMMARY

Microscopic examination of lung tissues often demonstrates metastatic carcinoma when grossly no tumor tissues are seen. The factors concerned with the implantation and metastatic growth of tumor cells are not understood.

The microscopic examination of the tissues of a woman, aged 44, whose primary carcinoma of the mammary gland had been treated

5. Hunter, J. B.: Brit. J. Surg. **15**:159, 1927.

6. Ciro, L., and Bolestra, G.: Pathologica **22**:451, 1930; abstracted, Cancer Rev. **6**:311, 1931.

surgically and with extensive roentgen irradiation, demonstrated a marked metastatic intracapillary and intravascular growth in the lungs. Similar less extensive intravascular metastases were in the spleen, adrenal glands, myocardium, vermiform appendix and ovaries. The carcinoma tissues were limited to the lumens of the blood vessels. They were not impacted masses but had grown into molded casts of the capillary channels. There was no extravascular growth in the lungs, spleen and vermiform appendix. In the ovarian and adrenal gland tissues the carcinoma had spread into the parenchyma.

General Reviews

HISTOLOGY OF THE THYROID IN EXOPHTHALMIC GOITER AND HYPERTHYROIDISM

C. ALEXANDER HELLWIG, M.D.
WICHITA, KAN.

CLASSIFICATION OF GOITER

Reading the Proceedings of the Second International Goiter Conference, held in Berne, Switzerland, in 1933, one is impressed with the large space devoted to the morphology of goiter. Almost every paper was illustrated with microscopic pictures of the diseased thyroid.

On the other hand, from the current American literature one gets the impression that biochemical study and animal experiment have replaced almost completely the morphologic method of investigation. However, even today the clinical pathologist has to describe, in his daily routine, the histologic changes found in removed goiters and has to give his opinion on their functional value. Postoperative prognosis and treatment depend largely on his histophysiologic diagnosis.

The lack of uniformity in the classification of thyroid changes is, without doubt, one of the reasons why there is such a disagreement among surgical pathologists in correlating structure and function of goiter. Although almost all European writers have accepted the classification of goiter by Aschoff, there is no uniform nomenclature in this country. The classification recommended by the American Association for the Study of Goiter may be satisfactory for the clinician, but it is certainly of no value to the pathologist. General acceptance of an international classification seems imperative. It would eliminate the present confusion, which hinges largely on words rather than on essential facts, and would allow a comparison of the various structures of goiter in different areas not only of this but of other continents.

CYTOLOGY AND HISTOLOGY OF THE THYROID IN ANIMALS

The pathologist who attempts to correlate the structure and the function of the diseased thyroid should familiarize himself first with the histophysiology of the thyroid in animals. Eggert's monograph, in which 327 original papers are critically reviewed, offers a wealth of information.

Epithelial Cell.—According to the functional phase of the thyroid, the form and height of the thyroid cells vary markedly. In the same

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Classifications of Goiter Used in English and German Literature

A. Kocher (1919)	Hellwig (1920)	Aschoff (1924)	Wegelin (1926)	McCarrison (1927)	Marine (1927)	Hertzler (1929)	Rienhoff (1929)	MacCarty (1931)
Struma diffusa	Hyperplasia	Diffuse goiter	Diffuse hyperplasia					Thyroid-shaped goiter
Struma congenita parenchymatosa	Congenital simple hyperplasia	Congenital diffuse goiter	Struma congenita neonati	Congenital goiter	Simple congenital goiter			
Struma hyperplastica follicularis	Microfollicular hyperplasia	Diffuse parenchymatous goiter	Struma diffusa parenchymatous microfollicularis	Parenchymatous goiter	Hyperplasia and hypertrophy			
Struma diffuse colloides	Macrofollicular hyperplasia	Diffuse colloid goiter (*a*) Without epithelial proliferation (*b*) With epithelial proliferation	Struma diffusa colloides macrofollic (*a*) Resting (*b*) Proliferant	Diffuse colloid goiter	Involution	Uniform stage of colloid goiter	Simple colloid goiter	Hypertrophic colloid goiter
Struma diffusa parenchymatosa papillaris	Exophthalmic goiter	Exophthalmic goiter	Struma diffusa basedowiana	Hyperplastic goiter	Exophthalmic goiter	Exophthalmic goiter	Hypertrophy and hyperplasia of the thyroid	Hypertrophic parenchymatous goiter
		Nodular hyperplasia	Nodular hyperplasia					
Struma nodosa	Adenoma	Nodular goiter	Adenoma					Nodular goiter
Struma nodosa parenchymatosa	Microfollicular adenoma	Nodular parenchymatous goiter	Parenchymatous adenoma	Adenoma	Nodular hyperplastic stage	Mixed tumor	True benign parenchymatous neoplasm of the thyroid	Adenomatous goiter with parenchymatous hypertrophy
Struma nodosa colloides	Macrofollicular adenoma	Nodular colloid goiter (*a*) Without epithelial proliferation (*b*) With epithelial proliferation	Colloid adenoma (*a*) Simple macrofollicular (*b*) Papillary macrofollicular	Colloid adenoma	Nodular involutionary stage	Bosselated stage of colloid goiter	Nodular colloid goiter	Adenomatous colloid goiter
Struma nodosa basedowificata	Adenoma basedowificatum	Nodular colloid goiter with marked epithelial proliferation	Adenoma basedowificatum	.....	Nodular goiter with hyperplasia	Chronic toxic stage of bosselated colloid goiter (toxic adenoma)	Nodular colloid goiter with hypertrophy and hyperplasia	

acinus, cells of different type are often encountered. The acinar epithelium is not uniformly low cuboidal, as generally accepted. One side of the follicle may be lined with a low, dark staining epithelium, whereas the cells of the opposite side are tall and light. The different height suggests different states of activity within the same follicle (Severinghaus).

Mitotic figures are present not only in the young but also in the adult animal during the active secretory phase. The cells are low, sometimes endothelial-like, when the secretion is sluggish, and they become higher and larger with increasing activity. While in flat cells the borders are indistinct, the high columnar cells have distinct granular borders. In the low cell the nucleus is oval and located at the basis; in the active cell it is large and vesicular and is situated in the center of the cell. Its diameter is between 5 and 6 microns during secretion (Uotila). Hirschlerowa found, during metamorphosis in the thyroid of the frog, a nucleocytoplasmic ratio of 60 per cent, as compared with 26 per cent in the resting gland. The nucleoli are largest during the resting phase and become smaller and less numerous with increasing activity. The significance of the nuclear changes in regard to the secretory process is little understood. The view held by Skowron that the nucleus plays a direct role in the formation of colloid is opposed by most investigators.

The formation of intracellular colloid is closely associated with fuchsinophilic granules, which are numerous in active cells. They were described by Galeotti in the turtle (1879), by Lobenhoffer in man (1907) and by Takagi in the dog (1922). Okkels, Severinghaus and Eggert expressed the belief that these granules are actual antecedents of colloid; they held that the granules become liquefied near the apical cell border, pass into the lumen of the follicle and are transformed into thin colloid. Thomas and Winiwarter expressed the opinion that these granules form the peripheral vacuoles of the colloid.

These prosecretion granules depend, according to Schultze, Takagi, Okkels and Uotila, on the catalytic action of the mitochondria. The latter take the form of granules, rods or filaments. The increase of mitochondria in size and number has been studied by Seecof in thyroid hyperplasia produced by a fat-rich diet. In guinea pigs he demonstrated their involution following administration of iodine. Exposure to cold also produced an increase in the number and size of the mitochondria in the thyroid of the rat and the mouse (Cramer and Ludford).

Severinghaus expressed the belief that fuchsinophilic granules and mitochondria are not related structures. During the production of colloid he saw the mitochondria decrease in prominence while the fuchsinophilic granules became abundant. During absorption of colloid the mitochondria appeared large in size and number. Thomas, studying

normal human thyroids, failed to observe a relationship between mitochondria and cellular activity. According to him, they change only their position in the cell during the different phases of thyroid activity.

Another cytoplasmic structure of great interest is the Golgi apparatus. There are conflicting opinions as to whether it can be seen in the living cell (Cramer and Ludford) or is an artefact due to special methods of fixation (Okkels). Cowdry was the first who studied these structures in the thyroid of the guinea pig. Usually he found the Golgi apparatus between the nucleus and the apex of the cell. Occasionally he saw it at the basis. He concluded that its position indicates the direction of secretion, either toward the lumen of the follicle or directly into the perifollicular capillaries. This view is supported by Ishimaru's observation that in hypersecretion numerous branches of the Golgi apparatus extend to the cell basis. On the other hand, Ingram, Alexandrov, Wagschal, Uotila and Uhlenhuth denied that the position of this structure indicates the direction of secretion. They agreed, however, that the Golgi apparatus becomes hypertrophic during secretory activity. Severinghaus and Ludford and Cramer showed that during active secretion droplets of colloid form in the reticulum of the Golgi apparatus. This observation and the fact that this apparatus hypertrophies only during secretion, not during resorption of colloid, seem to link the Golgi apparatus definitely with the new formation of colloid. One is able to say, by study of the Golgi apparatus, whether intracellular colloid droplets are the products of resorption or of newly formed secretion (Severinghaus).

Colloid.—In the normal thyroid the lumen of the follicle contains a homogeneous translucent substance which refracts light slightly more than water does. Its consistency varies from that of thin fluid to that of molasses. Occasionally the colloid is finely granular, even in normal glands (Ferguson; Eggert). The staining properties of the colloid and their functional significance have aroused much interest among students of goiter. Thin colloid stains red with eosin and blue with azocarmine, while thick colloid has an affinity to hematoxylin and stains red with azocarmine. To explain the acidophilic and basophilic staining reactions of the colloid, many theories have been ventured. It is generally accepted that freshly secreted colloid is acidophilic, old material basophilic. Hewer expressed the belief that colloid must be alkaline to be fit for resorption. On long storage it would become acid and inactive. However, exact determinations of the hydrogen ion concentration of different forms of colloid are lacking to substantiate this theory.

While most investigators accept only one chemical form of colloid, Kraus held that the thyroid cell secretes two different kinds, namely, fuchsinophilic and fuchsinophobe colloid. Wail also distinguished two

kinds of colloid. According to him, there are an acidophilic colloid, secreted by the cytoplasm, and a chromatin-rich "metanuclear" colloid, which is a product of nuclear substances liberated from desquamated acinar cells. Uotila stated that acidophilic colloid is secreted during the early phase of colloid storage. When the production of acidophilic colloid ceases, basophilic colloid continues to be secreted.

According to Kocher, the difference in the staining quality of the colloid depends on a change in its chemical composition, especially in its iodine content. Woitkewitsch showed that the thick colloid of resting thyroids is more effective in elevating the basal metabolism than is colloid obtained from activated glands. Schockaert found that acidophilic colloid is poorer in iodine than is basophilic. Wegelin believed that the different staining properties of the colloid are due not only to the degrees of concentration but also to chemical differences, for instance, differences in the content of calcium and organic iodine compounds. Carbohydrates, nucleinic acids, fats and lipoids seem also to play a role in the different staining quality, since these substances are present only in basophilic colloid.

Intracellular Colloid.—Chromophilic colloid is found not only in the lumen of the follicle but also occasionally in epithelial cells. Intracellular colloid droplets appear very seldom under normal conditions; they occur mostly only during increased activity. In only a few instances a large drop of colloid may be seen filling out the whole cell. This so-called Bensley cell may empty its content into the lumen of the follicle through the ruptured apical membrane and degenerate (Uhlenhuth). The secretion of stained intracellular colloid which passes into the lumen of the follicle is, according to Eggert, uncommon and does not constitute the normal production of colloid, as Florentin and Weiss held. The appearance of intracellular colloid droplets seems to indicate stagnation of colloid produced in the cell.

In 1916 Bensley advanced the theory that colloid secretion is normally directed to the capillaries. Only when the activity of the thyroid cell surpasses the body's needs for thyroxine, do colloid droplets become visible within the cell. The secretion produced by the cells condenses under these conditions, forms intracellular chromophilic droplets and is emptied into the lumen of the follicle. In the opinion of Grant, Okkels and Uhlenhuth, intracellular colloid droplets have an roid cell surpasses the body's needs for thyroxine do colloid droplets represent intrafollicular colloid which is resorbed by the cell and is on its way to the perifollicular circulation.

Vacuoles.—For a long time students of the thyroid have been interested in colorless vacuoles found in the periphery and in the middle of the colloid. These vacuoles are especially numerous in highly active

glands, while in the resting thyroid they are scanty or entirely absent. The peripheral vacuoles were described by Verson (1871) and Andersson (1894) in the fresh thyroid. Baber, Langendorff, Hürthle, Kocher and Alexandrov regarded them as artefacts, the result of shrinkage by the fixing fluid. Okkels stated that the large central vacuoles are due mostly to shrinking of the colloid, while the small ones, opposite clear hypertrophic cells of the wall of the follicle, are due to absorption of colloid. Uhlenhuth, Uotila and von Hagan interpreted them as a special kind of secretion of the epithelial cells, namely, the chromophobe colloid. Thomas and de Winiwarter held that the vacuoles contain the prosecretion of the chromophilic colloid. Most writers, including Aaron, Severinghaus, Guyénot, Ponse and Dottrens, contend that the peripheral vacuoles appear during the resorptive phase of thyroid activity and never occur in relation to low resting epithelium.

Occasionally one sees in the center of the colloid a single large vacuole which contains a fine granular coagulum, degenerated nuclei or cell fragments. Vrtel attributed the central vacuoles to proteolytic ferments liberated from cell fragments.

The intracellular vacuoles, which were described first by Andersson, are regarded by modern cytologists neither as artefacts nor as unstained colloid droplets but as resorbed intrafollicular colloid. It is, according to Uhlenhuth, transformed into an unstained liquid form in the cell and enters from there into the circulation. Klumpp and Eggert suggested that the intracellular vacuoles contain enzymes which transform the intrafollicular colloid into substances of lower molecular weight, thus enabling it to pass through the cell into the circulation.

While the normal procedure of colloid resorption is apparently by way of the epithelial cells, occasionally other mechanisms of colloid release are observed. Hermann, Florentin and Weiss, Vrtel and Hopkins stated that the colloid may pass into the circulation through clefts in the acinar wall caused by rupture or degeneration of thyroid cells. Another possibility was described by Eggert and Hertz. After stimulation of the thyroid by an extract containing the thyrotropic principle of the anterior lobe of the pituitary, they saw wandering cells invade the lumen of the follicle, take up colloid and transport it to the interfollicular tissue.

Cyclic Changes of Thyroid Activity.—Ferguson (1911) and especially Bensley (1916) expressed the opinion that the thyroid is a gland of reversible polarity. According to them, the secretion is normally produced in the thyroid cell, collects in colorless vacuoles at the basis of the cell and passes directly into the circulation. Only when the secretion is produced in excess of the body's requirements, does it appear as a condensed and stored product in the lumen of the follicle, i. e.,

as stained colloid. Cowdry and Ludford and Cramer supported this view by occasionally finding the Golgi apparatus in the basal portion of a cell. Okkels, Wahlberg, Uotila and Severinghaus, on the other hand, stated that during normal activity, when enough chromophilic colloid is present in the lumen of the follicle, colloid is resorbed from the lumen, passes through the cell and enters the capillaries. Only when the demand is excessive and the store of colloid becomes exhausted may secretion of colloid directly into the circulation take place.

From recent experimental studies by Uhlenhuth it must be concluded that not only in the normal but also in the hyperactive gland functional activity proceeds in cyclic waves of alternating storage and release of colloid. He studied the secretion process in the thyroid of the Californian newt by measuring the colloid level during different phases of activity. He found that even in glands stimulated by an extract containing the thyrotropic factor of the anterior lobe of the pituitary large quantities of colloid pass at times into the lumen of the follicle to be stored there instead of taking the more direct route from the cells into the circulation.

Knowledge of the histophysiology of the thyroid has been greatly advanced by studying the effect of glandular products on the thyroid. Administration of thyroid extract or of thyroxin decreases the activity of the thyroid, as demonstrated by Cameron and Carmichael, Gray and Rabinovitch, Kuschinsky and Eggert. The acinar epithelium becomes flat and the cell borders indistinct, and the cytoplasm contains only few fuchsinophilic granules. Andersson's vacuoles and intracellular colloid droplets are absent. The colloid is stored in the follicle as concentrated substance with few or no vacuoles. There is also decrease or complete absence of mitotic figures in the thyroid cells. The opposite effect is observed after injection of an extract of the anterior lobe of the pituitary gland containing the thyrotropic factor, which apparently is produced by the acidophilic cells. Its effect on the thyroid has been well established. It is the most dependable and fastest means of producing thyroid hyperfunction. Before the discovery of this factor, similar structural changes had been produced in the thyroids of experimental animals by exposure to cold, by a calcium-rich diet and by removal of large parts of the thyroid, leading to compensatory regeneration.

The effect of the thyrotropic factor of the pituitary gland has been studied in amphibians by Smith (1922), Spaul, Uhlenhuth and Schwartzbach; in ducks by Schockaert (1930) and in mammals by Smith (1927), Loeb and co-workers (1929), Aaron, Okkels and others. In all animals an enlargement of the gland occurs, with hyperplasia and hypertrophy of the epithelium, increase in the blood supply and emptying of the intrafollicular colloid.

Okkels studied the sequence of microscopic pictures at different intervals of time after injection of an extract containing the thyrotropic substance. Only thirty minutes after injection of this extract he was able to observe in the thyroid of the guinea pig the first definite histologic changes. The gland became hyperemic, and the cells appeared swollen. The nucleus and the few mitochondria were located at the basis, while the Golgi apparatus was not visible. About one hour after the injection the Golgi apparatus reappeared; it first formed fine filaments in the apical portion and finally developed into a thick network which occupied the entire area between the upper cell border and the nucleus. In this stage the intrafollicular colloid became vacuolated and was resorbed by the acinar cells. The peripheral vacuoles in the colloid were abundant, and many were in direct connection with the foamy cytoplasm of the cells. One hour after this stage the follicles were much smaller and the lumens more or less empty. The cytoplasm of the epithelial cells was filled with Andersson's vacuoles. It contained also long filamentous mitochondria, and the Golgi apparatus was markedly enlarged. This stage remained unchanged for at least thirty-six hours. Finally, the follicle filled again with colloid and the cells gradually returned to their normal form.

Okkels correlated these morphologic changes in the thyroid with functional changes. During the first hour after injection of the extract containing the thyrotropic substance the basal metabolic rate increased markedly and after a slight decrease remained constantly high during the observation until the follicle began to fill with colloid.

While Okkels depended on static pictures of thyroids from different animals, inferring the intermediate stages, a method was devised by Williams which made it possible to study the whole vital process by continuous observation. He attempted to solve the mystery of the direction of secretion, of the significance of the colloid and of the manner of its resorption by observing a single follicle in the living animal over a considerable period. In 1937 he installed a transparent Clark chamber in the ear of a rabbit. Autogenous transplants of the thyroid were placed in this chamber, and microscopic study of the living transplants was continued for sixty days.

By long periods of uninterrupted observation of a single follicle, Williams recognized four stages of a cycle: (1) secretion, (2) release of colloid, (3) partial collapse of the follicle and (4) recuperation. The first stage was characterized by roundness of the follicle and increasing refractibility of the acinar wall. In the second stage the refractibility of the wall increased while the thickness diminished. The chief mechanism of colloid release is, according to Williams, by diffusion through epithelial cells. When colloid release is faster than colloid production, the follicle collapses partially (third stage). Williams concluded from

his studies that secretion is toward the lumen of the follicle and that colloid release is through the acinar wall. Vacuoles that occurred at the periphery of the colloid he considered to be fresh secretion.

Injections of an extract containing the thyrotropic substance of the pituitary gland produced, in Williams' experiment, an exaggeration of the normal follicular activity with increased release of colloid in most follicles.

The newest method of biologic investigation, the cultivation of whole organs in the Lindbergh-Carrel pump, has been applied to the study of the thyroid. Glands from cats and rabbits transplanted into the organ chamber of this apparatus could be maintained alive for over a week. The perfused organs responded to modifications in the chemical composition of their medium by changes in their structure. The presence of the thyrotropic substance of the pituitary in the perfusing fluid was manifested by vacuolation of the colloid and by increase in the size of the thyroid cells, while the addition of iodine to the circulating fluid increased the amount of colloid in the perfused gland.

Structural changes as striking as those produced by injection of thyroxin or of an extract containing the thyrotropic factor of the pituitary seldom occur spontaneously in animals. The thyroid of a poikilothermic animal during hibernation resembles that of an animal receiving injections of thyroxin. On the other hand, during early metamorphosis amphibians have a hyperplastic thyroid which has characteristics of the effect of the thyrotropic factor of the pituitary.

As a rule, however, the secretory and resorptive processes, the pictures of rest and activity, occur simultaneously in the same thyroid. Different phases of the cyclic thyroid function may be present in the same follicle. The activity of the epithelium is seldom the same throughout the gland. This fact makes it difficult to determine the functional value even of a normal thyroid.

HISTOPHYSIOLOGY OF THE HUMAN THYROID

Interesting as these studies on thyroids of animals are, the question arises as to how far the results can be applied to the human thyroid. As compared with the large number of cytologic studies on thyroids of animals, the number of morphologic investigations of human thyroids by modern methods is extremely small. Two papers are of special interest, one from Belgium, the other from Finland. Goormaghtigh and Thomas made a survey of 500 human nongoitrous thyroids obtained at autopsies. In thyroids from patients with infectious and toxic conditions they found structures which are generally considered characteristic of toxic goiter. The epithelial lining of the follicles was not uniform, contrary to what is generally stated in textbooks. Often a narrow segment

of high columnar cells with dark nuclei was present in the acinar wall while the remaining epithelium was cuboidal or endothelial-like. There was a close relation between the extent of columnar epithelium and colloid release. According to Goormaghtigh and Thomas, columnar epithelium absorbs intrafollicular colloid and excretes an active hormone into the circulation. Colorless basal vacuoles were present only in this type of epithelium. The collapse of the wall of the follicle as a result of the release of colloid leads to formation of diverticula, which finally become separated from the main follicle. In this way, accessory small acini are formed which are lined with high cuboidal epithelium. When these small follicles fill up with colloid, each mass bulges into the lumen of the main follicle and produces a cushion-like elevation of its wall. This elevation is the so-called Sanderson polster of the literature.

This is, according to the Belgian writers, not a center of proliferation but a functional unit. It consists of a segment of columnar epithelium in a large follicle where colloid is resorbed and an underlying group of small acini where colloid is produced. In health and disease, these active functional units are ever changing structures.

Goormaghtigh and Thomas expressed the belief that it is not the proliferation of epithelial cells which determines the activity of a gland, but the extent of columnar epithelium. Microfollicular, parenchymatous goiters are just as rich in mitotic divisions and often attain larger size than colloid goiters. These parenchymatous goiters are not associated with hyperfunction because they are without excretory function, not having columnar epithelium. The fact that segments of columnar epithelium are frequently found in the large follicles of colloid goiter explains why this type of goiter is often accompanied with toxic symptoms.

Goormaghtigh and Thomas conclude from their study of 500 thyroids that the functional value of a given gland can be determined with great exactness, almost mathematically, by counting the number and measuring the extension of the columnar segments, i. e., the functional units.

The other paper which is based on modern cytologic methods is that by Wahlberg. His material consisted of only 2 normal thyroids and 15 goiters, but from every specimen 22 to 42 blocks, from different parts of the gland, were sectioned. Eight different methods of fixation and thirteen different staining methods were employed. For the study of finest cell structures, sections from 1 to 2 microns thick were selected. Wahlberg's remarkable monograph is illustrated with 102 beautiful photomicrographs. His investigation demonstrated these facts: All secretory activity of the thyroid is characterized by cellular hypertrophy, whereby low cuboidal epithelium of the resting gland becomes columnar. If the activity becomes excessive, there is also epithelial proliferation.

The increase in the number of cells results in the development of infoldings of the follicle wall, varying from flat elevations to complicated papillae. Wahlberg distinguished two phases of thyroid function, the storage of colloid and its resorption. Both can be recognized microscopically by special types of proliferation, namely, colloid-producing proliferation. and hormone-resorbing proliferation. What Wahlberg designated colloid-producing proliferation is the same cushion-like elevation of the follicle wall which is generally known as Sanderson's polster. Wahlberg's view that the cells of this columnar segment secrete colloid into the follicle is in disagreement with that of Goormaghtigh and Thomas, who interpret them as units connected with colloid resorption. The hormone-resorbing proliferation of Wahlberg is represented by follicles in each of which the whole wall consists of activated columnar cells. The latter provide hormone by resorption of intrafollicular colloid but mostly by direct secretion of thyroxine into the capillaries without previous storage. In Wahlberg's opinion, most of the cells in the normal thyroid are inactive. The relative numbers of the two functional units—colloid-producing and hormone-resorbing proliferation—determine the functional value of a gland.

In colloid goiter the hyperplasia is of the same type as the colloid-forming proliferation of the normal thyroid, only more extensive in size and number. In toxic goiter the number of hormone-resorbing proliferations is markedly increased, either throughout the gland or in circumscribed areas. In these units the polarity of the cells is reversed. The colloid is absorbed from the lumen of the follicle through the apical cell border, passes through the cytoplasm in the form of droplets, collects with fresh secretion in large vacuoles at the basis and is excreted either directly or through intercellular spaces into the capillaries. According to Wahlberg, most of the hormone is formed in the cytoplasm and excreted directly into the circulation without intrafollicular storage. Wahlberg often observed, during active basal secretion, degenerative processes in the columnar cells. He expressed the belief that autolytic cell products are liberated and that the change in the chemical composition of the hormone accounts for the dysfunction of toxic goiter.

Every increase in the activity of the thyroid was accompanied with hypertrophy of the mitochondria and of the Golgi apparatus. These structural changes were observed regardless of the direction of secretion. During excessive activity the Golgi apparatus was swollen and ramified. It was always situated in the apex and did not change its position during reversal of the polarity. Since all these cytologic changes were identical, whether the toxic goiter was diffuse or adenomatous, Wahlberg regarded the latter as clinical and pathologic entities.

With administration of iodine the active cells change their direction of secretion from basal to apical. There is some epithelial involution;

however, the mitochondria and the Golgi apparatus remain hypertrophic. This fact explains why the clinical improvement is only transitory. During iodine treatment the gland is also continuously under the influence of an activating extrathyroidal impulse. After discontinuance of the iodine medication the toxic symptoms often return in more severe form because the lumens of the follicles have been filled with highly active colloid.

Feyel and Varangot studied 5 normal thyroids and 7 simple and 21 exophthalmic goiters with modern cytologic methods. They confirmed the observation by Thomas that there are four well characterized types of epithelium in the normal and in the diseased thyroid: Columnar epithelium, which resorbs the hormone and discharges it into the circulation; high cuboidal epithelium, which produces colloid very actively; low cuboidal epithelium, which manufactures colloid slowly, and endothelium-like flat epithelium, which is inactive.

In exophthalmic goiter the two authors found only the first two cell types. Even the most modern cytologic staining methods failed to reveal any structural differences between cells of the same type in normal and, on the other hand, diseased thyroids. Basal vacuoles were found only in the columnar cells. Apical small vacuoles, which in the opinion of Feyel and Varangot are a part of the Golgi apparatus, could be demonstrated with Cajal's silver stain. Their size and number are a reliable criterion of cellular activity.

GEOGRAPHIC PATHOLOGY OF TOXIC GOITER

The first two days of the Second International Goiter Conference were devoted to the structual changes of the thyroid in hyperthyroidism and in exophthalmic goiter. It was the first occasion to discuss this problem from a world-wide standpoint. The principal speaker was de Josselin de Jong, who reported clinical and pathologic studies of 1,050 goiters removed by operation in Utrecht, Holland. Of the patients, 113 (15.5 per cent) had hyperthyroidism, and 123 were considered clinically to show the syndrome of exophthalmic goiter. Hyperthyroidism is caused, in de Josselin de Jong's opinion, by hyperactivity of the thyroid and hypersensitivity to thyroxine, while the clinical condition known as exophthalmic goiter is a constitutional disease in which the disturbance of thyroid function is an important factor but in which the nervous system, other endocrine glands and the lymphatic system are involved also.

Of the specimens from patients with the clinical syndrome of exophthalmic goiter, 76 per cent showed the histologic picture of exophthalmic goiter. In 13 per cent de Josselin de Jong found diffuse colloid goiter and in only 11 per cent nodular goiter. Without preoperative

administration of iodine the typical exophthalmic goiter presents the following structure:

1. There is marked variation in form and size of the follicles. The epithelium is irregular—as a rule, columnar. The nuclei are closely packed and situated at the basis of the cells. They are rich in chromatin and vary in size. Hypertrophy of the Golgi apparatus is demonstrated by silver staining methods. There is increased desquamation of the epithelium.

2. The amount of colloid is markedly decreased. The colloid is thin and often vacuolated. It takes eosin only lightly or not at all.

3. Groups of lymphocytes, often with distinct germinal centers, are frequently found (in 71 per cent of de Josselin de Jong's exophthalmic goiters).

According to de Josselin de Jong, the pathologist is justified in making a diagnosis of exophthalmic goiter if he finds these three structural changes in a diffuse goiter.

The introduction of preoperative medication with iodine has markedly changed the gross and the microscopic picture of the thyroid of the patient who shows the syndrome of exophthalmic goiter. In 68 thyroids from patients treated with iodine de Josselin de Jong noticed as an outstanding characteristic the abundance of stained colloid. Not all follicles, however, were filled with colloid. In many exophthalmic goiters there were found, besides large colloid-filled follicles, areas with small acini lined with columnar epithelium and with unstained colloid. Lymphoid tissue was found after iodine treatment as frequently as when there had been no iodine medication. Iodine did not cause complete involution of the gland; hyperplastic areas were always still present. The Golgi apparatus remained large in the epithelial cell.

The structural changes in the thyroid of the patient with the syndrome of exophthalmic goiter are interpreted by de Josselin de Jong as due not only to hyperplasia and hyperfunction but also to lack of apical secretion of colloid. The inability of the exophthalmic goiter to synthesize and store intrafollicular colloid is evident. Most of the secretion formed in the cytoplasm is apparently discharged directly into the circulation without previous storage in the follicle.

De Josselin de Jong found 41.6 per cent of the goiters associated with hyperthyroidism to be diffuse colloid goiters. There were only half as many nodular colloid goiters. The microscopic picture of colloid goiter, with and without hyperthyroidism, is seldom characteristic enough to make a functional diagnosis. In diffuse colloid goiter associated with toxic symptoms de Josselin de Jong found the colloid as a rule more liquid and the epithelium slightly higher, and he found

lymphoid tissue in such goiters twice as often as in simple goiters. Toxic nodular goiter did not show any characteristic histologic changes to distinguish it from nontoxic nodular goiter. On the other hand, many innocent goiters may present, especially during adolescence, small hyperplastic areas which resemble those found in toxic goiter. Ninety-five per cent of the patients with hyperthyroidism gave a history of long-standing innocent goiter, while exophthalmic goiter was primary in 80 per cent. The latter, in de Josselin de Jong's opinion, is not related to endemic goiter.

Well circumscribed adenomas were found in 11.2 per cent of the goiters from patients with hyperthyroidism, and in 3.2 per cent of those from patients with the syndrome of exophthalmic goiter. These tumors were mostly single, had a radial structure and showed a tendency toward degeneration. Only 5.5 per cent of all toxic goiters were of this type. A diagnosis of toxic adenoma should be limited to the following characteristics: 1. The enlargement of the gland should be due entirely to the presence of one single well encapsulated parenchymatous adenoma or several. 2. The toxic symptoms should disappear after enucleation of the adenoma.

In de Josselin de Jong's experience, a functional diagnosis of toxic goiter cannot be made by special stain of the colloid. He stained a number of toxic and nontoxic goiters with azocarmine, following the method of Troell. Not only in different sections of the same goiter but also in the same section he found red and blue-stained colloid. The statement made by Troell, Kraus and Jones that colloid in toxic goiters gives a specific staining reaction could not be confirmed.

While lymphoid tissue is frequently found in exophthalmic goiter, its presence is not always a criterion of thyroid hyperfunction. In de Josselin de Jong's material 17 per cent of nontoxic goiters and 38 per cent of goiters associated with hyperthyroidism had lymphoid tissue. Often it was found in the tissue surrounding adenomas, apparently as an inflammatory reaction caused by degeneration of thyroid cells.

At the same conference, Roussy, Huguenin and Welti discussed the structure of 138 toxic goiters found in Paris. All of their 77 specimens from patients with the syndrome of exophthalmic goiter showed specific thyroid changes. In no other disease does the thyroid show these characteristics. Two types of exophthalmic goiter are distinguished by the French authors. In the first type the gland is lobulated, vascular and sclerotic. The colloid is absent or very scanty, and the connective tissue is increased. This is the so-called cirrhotic form of exophthalmic goiter. The second type is characterized by a moist cut surface. There are many papillary projections of the wall of the follicle, protruding into the lumen. In this type lymphoid tissue is

relatively rare. Often, large polyhedric cells which stained intensively with eosin were found. They were regarded as degenerative thyroid cells.

There was no difference in severity or duration of the clinical symptoms between these two different types of exophthalmic goiter. The effect of iodine on exophthalmic goiter was studied by Roussy and co-workers in 28 cases. The accumulation of colloid was striking and seemed to depend on the duration and dosage of the iodine given before operation. However, even after large doses, high columnar cells and proliferations of the follicle wall were often found together with large colloid-filled follicles. In several cases no correlation was found between histologic regression and improvement of the toxic symptoms.

Secondary toxic goiters, developing in long-standing simple goiters, are common in Paris. The toxic goiters were either diffuse or nodose. The nodules varied in size and number. The histologic picture resembled that of exophthalmic goiter after iodine treatment. Proliferative areas with thin colloid, columnar epithelium and lymphoid tissue were found either in small circumscribed patches or throughout the gland. Large areas of the toxic goiters presented the characteristics of colloid goiter.

In simple colloid goiter areas of epithelial proliferation are not uncommon. Roussy and co-workers believe that these hyperplastic changes are very probably responsible for an increased activity of the endemic goiter in Paris, where it is often associated with mild toxic symptoms.

In the material of the French authors were 39 toxic goiters which were removed after intensive treatment with roentgen rays. The most characteristic changes were sclerosis of the gland, especially near the surface, endarteritis with proliferation of the intima, and focal necrosis of the parenchyma.

In conclusion Roussy stated that in toxic goiter there is no close relationship between the structural changes and the severity of the clinical findings. The presence or absence of certain clinical symptoms, such as exophthalmos, cannot be diagnosed from study of the microscopic sections. There will always be a number of toxic goiters in which it is impossible to establish a correlation between structure and function. Only the syndrome of exophthalmic goiter is associated with morphologic changes in the thyroid which are specific.

Wegelin agreed with de Josselin de Jong that the diseases known as exophthalmic goiter and hyperthyroidism are distinct clinical and pathologic entities. The thyroid of the patient with the syndrome of exophthalmic goiter who has not been treated with iodine presents, according to Wegelin, such a characteristic morphologic picture that a

clinical diagnosis can be made in almost every case from the microscopic slide. Secondary toxic goiter and toxic adenoma, on the other hand, are not associated with a typical histologic picture. One may find there proliferative areas which resemble exophthalmic goiter; however, it is not safe to make a functional diagnosis from these findings, because the same epithelial hyperplasia may be found in simple colloid goiter, especially during adolescence. Toxic goiter following iodine treatment (*Jodbasedow*), which clinically may resemble primary exophthalmic goiter, often presents no morphologic evidence of hyperactivity.

De Quervain reported his unique experience with *Jodbasedow*. He removed, during nine years, 33 goiters which had developed after iodine medication. All his patients had severe toxic symptoms and 15 had exophthalmos. The basal metabolic rate was, in the average, 39 per cent above normal. Twenty-six of the goiters had single or multiple nodules. These nodular goiters were very rich in colloid, while in general the endemic goiter in Berne has a very small amount of colloid. According to de Quervain, there are two possibilities to explain this fact: Either colloid-rich goiters are more liable than parenchymatous to become toxic, or iodine medication transforms colloid-poor, parenchymatous tissue into colloid-rich tissue. Histologic study of these goiters suggested that not only the storage of colloid was markedly increased, but also its absorption. In contradistinction to the statement of Boothby that iodine never causes toxicity in diffuse goiters, de Quervain found among his goiters from patients with *Jodbasedow* 7 diffuse goiters, namely, 5 typical exophthalmic goiters and 2 diffuse colloid goiters with epithelial proliferation.

The histologic nature of toxic goiter as found in Basel, Switzerland, at the periphery of the Swiss goiter region, was described by Merke. He distinguished three different forms of thyrotoxicosis, according to clinical and structural characteristics. In his experience exophthalmic goiter has a specific histologic picture, presenting long narrow irregular follicles with high columnar epithelium, scanty or unstained colloid and low iodine content. After iodine medication the colloid is increased, the epithelium becomes lower and the iodine level is markedly raised. The secondary toxic goiter of Merke's classification is anatomically a diffuse or, more often, a nodular colloid goiter with small areas of hyperplasia which resemble exophthalmic goiter. Colloid and iodine are much higher in this type than in primary exophthalmic goiter since there is always a great amount of colloid-rich tissue present. From these two forms Merke distinguishes a third type of hyperactivity, which he designates "struma toxica." It is found in the older patient who has had an innocent goiter for many years and slowly comes to have single thyrotoxic symptoms. The outstanding symptom in this group is cardiovascular degeneration. The basal metabolic rate is increased. Histo-

logic examination of these goiters fails to reveal epithelial hyperplasia; on the contrary, most of the nodules are markedly degenerated. Only in few areas one may find evidence of epithelial proliferation and colloid resorption. These changes are, however, not pathognomonic, since they are found just as often in goiters from patients without any toxic symptoms. The iodine content of these goiters is extremely high, and preoperative administration of iodine is of no benefit.

The frequency of toxic adenoma in Sweden was the subject discussed by Holst. Among his toxic patients only 3 or 4 per cent presented this type. The diagnosis of toxic adenoma should be confined to those patients who are cured of their toxic symptoms within two weeks after enucleation of the adenoma. The pathologist is unable to make a diagnosis of toxic adenoma from the microscopic sections. The adenoma may be parenchymatous, colloid rich or cystic. In most cases which Holst observed the toxic adenoma developed in long-standing nodular goiter after iodine treatment. The iodine content of this type of goiter was always very high.

In 1931 Arndt published an interesting monograph on goiter in Russia. It is based on the histologic examination of 376 surgically removed goiters and 830 thyroids obtained at autopsies. Geographically this material covers regions of nonendemic and regions of endemic goiter, the vast plains as well as the mountains of this immense territory. In goiter-poor level regions—e. g., Moscow, Minsk, Kharkov and Leningrad—Arndt found as the most common type the diffuse colloid goiter with epithelial proliferation. In adults it was, as a rule, accompanied with toxic symptoms. Primary exophthalmic goiter was only half as common as the secondary form. The latter developed in diffuse, less often in nodular, colloid goiter. Lymph follicles were present in 71 per cent of all toxic goiters and in 90 per cent of primary exophthalmic goiters. Arndt stated that the large number of diffuse colloid goiters in his material of toxic goiters cannot be attributed to the preoperative administration of iodine, because at this time very few surgeons in Russia used Plummer's preoperative treatment.

The histologic aspects of exophthalmic goiter in Danzig were studied by Neumeyer (1937). This region at the Baltic Sea has a high frequency of endemic goiter in spite of a high content of iodine in food, water and air. Among 84 goiters removed by operation Neumeyer found 20 primary and 16 secondary exophthalmic goiters. The average weight of the resected goiters was between 30 and 40 Gm. Only a single patient with the syndrome of exophthalmic goiter had a nodular goiter. Microscopically the size and form of the follicles varied markedly, the epithelium was high columnar, and there were many papillary proliferations and cushion-like elevations of the wall of the follicle. After administration of iodine the amount of intrafollicular colloid was mark-

edly increased, and the papillary proliferations were less extensive. In 5 of the 20 primary exophthalmic goiters desquamation of the epithelium was noticed. In all exophthalmic goiters Neumeyer found lymphoid tissue, and in 5, typical follicles with germinal centers. The three constant morphologic characteristics of the thyroid of the patient with the syndrome of exophthalmic goiter are, according to Neumeyer, epithelial proliferation, liquefaction of the colloid and infiltration with lymphocytes. Secondary toxic goiter was either diffuse or nodular and always rich in colloid. There were, as a rule, hyperplastic islands with columnar epithelium and often papillary or cushion-like elevations of the wall. In 3 toxic goiters lymphocytic infiltration was the only morphologic sign of hyperactivity. Administration of iodine had no histologic effect on secondary toxic goiter.

From his clinical and pathologic studies Neumeyer concluded that in the region of Danzig a diagnosis of exophthalmic goiter can be made with certainty from the microscopic study of the thyroid.

Mayer and Fürstenheim studied 25 cases of the syndrome of exophthalmic goiter and compared the clinical and histologic observations very carefully. Their material was obtained in Berlin. In these 25 cases they found only 4 goiters with columnar epithelium, papillary epithelial proliferation and unstained colloid. Of the patients from whom these 4 goiters had been removed, the first had exophthalmos, the second severe thyrotoxicosis without ocular signs, the third mild symptoms of hyperthyroidism and the fourth no toxic symptoms. The latter had been cured of his symptoms by operation several years before.

Twenty-five patients had very severe toxic symptoms; of these, 3 had typical thyroid changes with columnar epithelium, papillary proliferation and scanty colloid; 5 had diffuse colloid goiter with marked epithelial proliferation; 9 had a few, and 8 had no epithelial proliferation. Mayer and his co-worker did not find a correlation between the extent of the hyperplasia and the severity of the toxic symptoms. On the other hand, papillary proliferations were present only in toxic goiters. It was impossible to determine from the histologic picture whether the patient had exophthalmos or not. Two patients with exophthalmos, for instance, had diffuse colloid goiters without epithelial proliferation.

Mayer and Fürstenheim found in the staining reaction of the colloid goiter a better histophysiologic criterion than in the epithelial changes. They employed the method of Kraus (methylene blue–tannic acid–fuchsin) and that of Mallory (aniline blue–gold orange). In their 22 nontoxic goiters the colloid stained red with Mallory's method and blue with Kraus's method. In the 25 toxic goiters most of the colloid stained blue with the first method and red with the second. There were only 2 exceptions among the toxic and 5 among the nontoxic goiters.

The high incidence of colloid-rich goiters in their series of toxic goiters was not due to administration of iodine, since only about half of the patients were treated with iodine previous to the operation. Mayer and Fürstenheim conclude from their study that the extent of columnar epithelium in a toxic goiter is not a reliable criterion of the severity of clinical symptoms. Smooth-walled follicles with low elevations may be found in cases in which the symptoms indicate great toxicity. They recommended special stains of the colloid for a functional diagnosis of goiter sections.

Orator and Schleussing studied normal thyroids and surgically removed goiters as found in the lower Rhine Valley, from the morphologic, chemical and clinical points of view. Of the 80 patients whose goiters were studied, 17 presented the syndrome of exophthalmic goiter. The goiters of 10 of these were typical exophthalmic goiters, 5 were diffuse colloid goiters with epithelial proliferations, and 2 were nodular colloid goiters. Fourteen goiters were of the secondary exophthalmic type. Anatomically, this group consisted of 10 diffuse goiters, 3 diffuse-nodular goiters and 1 nodular goiter.

Of the 7 diffuse colloid goiters with epithelial proliferation, 5 were associated with severe symptoms of exophthalmic goiter while 2, which occurred in adolescent girls, were nontoxic. The two investigators held that a definite correlation between histologic changes of the thyroid and clinical symptoms is lacking in many cases. Colloid-rich goiters with proliferation are, in their opinion, closely related to the development of thyrotoxicosis.

In 1931 Bürkle–de la Camp compared clinical and histologic observations on 400 goiters. His material was obtained in the southern part of Germany. No preoperative iodine treatment had been used. In adults the diffuse colloid goiter with areas of hyperplasia was often associated with mild or moderately severe hyperthyroidism. Two patients with symptoms of exophthalmic goiter had the same form of goiter. In adolescents the diffuse colloid goiter with the same degree of hyperplasia was not accompanied with toxic symptoms. Nodular colloid goiter with epithelial proliferation produced only mild toxic symptoms; often it was found without any signs of hyperactivity.

It is interesting to compare the results of Bürkle–de la Camp with those obtained by Spatz in the same region three years previously. The latter's material consisted of only 52 goiters, but his analysis was much more thorough since he not only examined many blocks from different parts of the goiters histologically but also determined the iodine content and the biologic effect on the metamorphosis of tadpoles. Spatz found typical microscopic pictures only in the primary and the secondary exophthalmic goiter, together with a low iodine content and a high biologic effect. The histologic changes of goiters associated with hyper-

thyroidism resembled somewhat exophthalmic goiter, but it was often impossible to differentiate between simple endemic and toxic goiter by the microscope. The effect of goiter tissue on the metamorphosis of tadpoles proved, in his series, to be more reliable in evaluating the activity of the thyroid than histologic study. There was no definite correlation between the severity of toxic symptoms and the histologic picture, the iodine content and the biologic effect.

The 500 goiters which were studied by Feriz had been removed by O. Lanz in Amsterdam. In his opinion the severity of the toxic symptoms is paralleled by the degree of epithelial proliferation. Alterations in the epithelial lining of the follicles were better criteria in a functional diagnosis than the color and concentration of the colloid. Feriz did not attribute any importance to changes in the interfollicular tissue. In his series the simple colloid goiter was nontoxic, the colloid goiter with epithelial proliferation was associated with hyperthyroidism, and the parenchymatous exophthalmic goiter was found associated with the syndrome of exophthalmic goiter. Feriz stated that a clinical diagnosis is feasible on the basis of histologic observations as far as the diffuse goiter in the adult is concerned. Epithelial desquamation is, according to Feriz, a degenerative process which is common in exophthalmic goiter. Lymphocytes are found mostly in degenerative areas. They are apparently attracted by substances liberated from autolytic cells.

In a review of the pathologic changes in the thyroid in 910 cases of hyperthyroidism, Rienhoff and Lewis stated that in all cases in which the patient was toxic they found hypertrophy and hyperplasia of the epithelium. A sufficient number of sections have to be cut from different regions of the gland to find the typical epithelial changes in some cases. In exophthalmic goiter without previous iodine treatment the follicles were irregular in shape and size, and the colloid was diminished and stained very slightly. The colloid was separated from the epithelial cells by unstained vacuoles. The cells were columnar, and there was infolding of the follicular wall. The cytoplasm of the epithelial cells contained not only many mitochondria but colorless vacuoles, and the nucleus was situated at the base of the cell. Following preoperative administration of iodine, Rienhoff found the colloid abundant, less liquid and well stained with eosin. The infolding of the follicular wall was smoothed out; the epithelial cells were shrunken and the nuclei pyknotic.

In some areas there were colloid-filled cysts and circumscribed groups of dilated follicles. Rienhoff expressed the opinion that these colloid cysts and nodules were due to iodine and that, as a rule, the nodular colloid goiter develops by involution from a hyperplastic gland.

He found among 109 toxic nodular goiters only 8 per cent true adenomas and denied that a neoplastic nodule could produce toxic symptoms.

In 1929 Rienhoff studied the normal and the hyperplastic thyroid by the wax plate reconstruction method. He demonstrated in exophthalmic goiter an enormous increase in the size of the follicles. The shape varied from a perfect sphere to a long narrow tube; the size ranged from 0.02 to 0.9 mm. Small follicles situated around a large follicle were shown to be distinct individual acini and not diverticula of the epithelial wall of the large follicle. The only papillomatous irregularities of the wall were those observed inside, in the lumen of the follicle.

The histologic aspects of the hypertrophic parenchymatous thyroid, i. e., exophthalmic goiter, were studied on the abundant material of the Mayo Clinic by Broders in 1936. Without administration of iodine the exophthalmic goiter was composed of round, oval or irregular follicles lined with cuboidal to high columnar epithelium. There were, to a large extent, papillary infoldings of the follicular wall and a small or moderate amount of colloid of varying density. After the advent of preoperative administration of iodine, Broders found the epithelium changed from columnar to cuboidal and the acini more dilated and containing more and deeper-stained colloid.

In Broders' material some exophthalmic goiters were nodular, the nodules usually presenting the same epithelial changes as the diffuse part of the gland. A number of specimens had true adenomas with columnar epithelium and more or less colloid; others had fetal acini or small undeveloped follicles lined with cuboidal epithelium and containing little or no colloid.

Broders found in all forms of exophthalmic goiter varying degrees of chronic thyroiditis, characterized by fibrosis and lymphocytic infiltration of the interfollicular tissue with or without formation of germinal centers. In his opinion these changes represent an inflammatory reaction to toxic products of the goiter. Because they had been much more common since the preoperative use of iodine, Broders suggested that iodine may activate this form of thyroiditis. In 9 cases he found single or multiple pink or white areas at the cut surface of exophthalmic goiters. These areas contained cells which varied in size from that of a plasma cell to that of a tumor giant cell. Broders expressed the opinion that these cells were regenerative and hyperplastic. Apparently they were cells of the type described by Roussy and MacCallum in exophthalmic goiter as due to degeneration. According to Broders, one cannot distinguish the toxic from the nontoxic adenoma macroscopically, nor can one always do so microscopically. However, when one sees an adenoma in which columnar epithelium is prominent, it is safe to consider it a toxic adenoma, the degree of toxicity probably depending on the amount of columnar epithelium and on the size and number of such adenomas.

In order to make a microscopic diagnosis of toxic adenoma, the columnar epithelium, occasionally associated with a papillary infolding, must be limited to the adenoma. In other words, the surrounding thyroid tissue proper should not show the microscopic features of exophthalmic goiter. Harms compared clinical and histologic observations in 200 cases of goiter in which operation was done in Wisconsin. There were 96 patients with hyperthyroidism and 50 with the symptoms of exophthalmic goiter. Of the 37 patients whose condition was clinically diagnosed as primary exophthalmic goiter, only 15 showed the typical microscopic picture with columnar epithelium and scanty colloid, while the majority (21) presented diffuse colloid goiter with epithelial proliferation. Three colloid nodules with extensive papillary proliferation were found in patients who had typical symptoms of exophthalmic goiter. Hyperthyroidism was associated with diffuse goiter in 52 patients and with nodular goiter in 44. In this group only 2 fetal adenomas were found; the other goiters were all composed of large colloid-filled follicles, often with epithelial proliferation. Most of the nontoxic nodular goiters were nodular colloid goiters without epithelial proliferation; however, 5 showed some areas of hyperplasia.

The functional-structural relationship in 544 goiters removed in Brooklyn was studied by Rabinovitch, Pearson and Louria. Of the patients, 294 had symptoms that were diagnosed clinically as those of exophthalmic goiter, 50 were considered to have toxic nodular goiter, and 200 were classified as having colloid and nodular nontoxic goiter. Of the 294 thyroids from patients with the syndrome of exophthalmic goiter, 222 (75.5 per cent) showed definite anatomic evidence of increased activity. The epithelium was columnar; many mitotic figures were found; the colloid was thin and scanty and the stroma vascular. The epithelial wall of the follicle in many instances formed spurlike projections into the lumen. Lymphoid tissue was a frequent finding. The remaining 72 thyroids from this group showed relatively little gross or microscopic evidence of hyperplasia, although clinically the patients presented severe symptoms of thyrotoxicosis with exophthalmos. The nodular toxic goiters numbered 50, and in 84 per cent of the patients there was a marked relationship between the clinical and the anatomic picture. Clinically the patients presented evidence of a moderate degree of thyrotoxicosis with no ocular signs. Anatomically the glands appeared to consist of multiple nodules with definite hyperplastic changes. The colloid and nodular nontoxic group numbered 200, and 92 per cent of the patients presented a definite relationship between the anatomic and clinical findings. In this group the patients presented few toxic symptoms, and the basal metabolism was relatively low. The glands showed all degrees of degenerative changes, such as fibrosis, cyst formation, hemorrhage and calcification. Active hyperplasia was rarely encountered.

The acini in the colloid goiters were very large and distended; the epithelium was very low. In the nodular type the acini were irregular in size and shape, with the epithelium low or flattened, often undergoing extensive degeneration.

Rabinovitch, Pearson and Louria concluded from their studies that there exists a certain parallelism between the structure and the function of the thyroid gland. This relationship, according to them, is less apparent in exophthalmic goiter but is very pronounced in the other forms of goiter.

In 1930 Simpson pointed out the inability to reconcile the clinical manifestations of hyperthyroidism with the pathologic changes in goiters. Especially, since the use of iodine became general, it is, according to Simpson, often impossible to adjust the pathologic observations to the clinical symptoms on a basis of epithelial changes alone. He expressed the belief that the presence of lymph follicles is much more constant and therefore a much more reliable criterion for hyperthyroidism than epithelial proliferation. In a series of 265 exophthalmic goiters and 121 toxic nodular goiters, epithelial hyperplasia was absent in 95 specimens, whereas lymph follicles were present in all. He accepted Warthin's thesis that in all cases exophthalmic goiter and toxic nodular goiter possess an identical underlying constitutional abnormality, the thymicolymphatic diathesis, and that lymph follicles in a thyroid are a never failing sign of "Graves' constitution."

In 1929 I studied the structure and function of 151 goiters from the Hertzler Clinic in Halstead, Kan. Of the 9 diffuse colloid goiters without epithelial proliferation, 3 were nontoxic, and 4 were associated with mild and 2 with severe hyperthyroidism. Exophthalmos was absent in the patients from whom these 9 goiters were removed. Nineteen diffuse colloid goiters with epithelial proliferation were found in this material. Four young girls with this type of goiter did not present toxic symptoms, while of the adults, 2 were considered to have the syndrome of exophthalmic goiter and 12 hyperthyroidism. There was in this group only 1 adult who did not show evidence of increased thyroid function. By histologic examination it was shown that 35 of the 57 exophthalmic goiters developed in diffuse colloid goiters; 74.4 per cent of the patients with these secondary exophthalmic goiters had exophthalmos; 7 had no ocular signs, and 2 had only mild symptoms of hyperthyroidism. The 22 patients with primary exophthalmic goiters had exophthalmos in about the same incidence, namely, 77.2 per cent. All patients in this group had most severe toxic symptoms.

In 5 of my specimens nodular hyperplasia, the transitional stage between diffuse and nodular colloid goiter, was found. Only 1 of the 5 goiters was nontoxic. Of the 21 parenchymatous (fetal) adenomas, 3 were associated with toxic symptoms. Nodular colloid goiter, on the

other hand, was often the cause of hyperthyroidism. There were in my series, 24 nodular colloid goiters without and 20 with epithelial proliferation. The average age of the patients was 48.4 years. The first toxic symptoms were noticed about fifteen years after the neck became enlarged. Twelve of the 24 nonproliferant nodular colloid goiters were associated with hyperthyroidism; the symptoms were severe in 5 patients, but no exophthalmos was found in this group. Nodular colloid goiter with epithelial proliferation was found in 13 patients, and 11 of those had definite toxic symptoms without exophthalmos. In 7 nodular colloid goiters columnar epithelium and papillary elevations of the wall of the follicle were noticed in the peripheral areas of the nodules. Five of these 7 goiters were from patients whose condition had been diagnosed by the clinician as severe hyperthyroidism; 1 was from a patient who was considered to show the syndrome of exophthalmic goiter.

From this study of surgically removed goiters in Kansas, the following conclusions are justified: Two thirds of the diffuse colloid goiters without epithelial proliferation and four fifths of the diffuse colloid goiters with epithelial proliferation are associated with symptoms of hyperthyroidism. The latter type may produce, in rare instances, the complete clinical picture of exophthalmic goiter. Fifty per cent of nodular colloid goiters without epithelial proliferation and 85 per cent of nodular colloid goiters with epithelial proliferation are associated with signs of thyrotoxicosis. The latter form of goiter may, in exceptional cases, produce exophthalmos. The single parenchymatous adenoma (fetal adenoma) plays a very small role among the toxic goiters, the incidence being not more than 4 per cent.

According to Hertzler (1937), there is no more constant relationship between pathologic change and clinical symptom and sign in all the rest of the field of surgical pathology than in exophthalmic goiter. Never once had he failed to find the characteristic histologic changes if the clinical syndrome of exophthalmic goiter was present. He distinguished two forms of this disease. The exophthalmic type, with ocular signs, is characterized in the microscopic section by papillary hyperplasia, and the nonexophthalmic type, by follicles without papillae. In the latter the acini may be so small that there are few or no lumens. Generally speaking, he found the histologic changes in proportion to the clinical manifestations of the disease. The greater part of the goiter from the patient with the symptoms of exophthalmic goiter may show simple colloid goiter. Adequate study will, however, always, even after iodine treatment, show cellular hyperplasia in some foci if the clinical diagnosis of exophthalmic goiter is correct. The toxic nodular colloid goiter is, according to Hertzler, the most common form of goiter. It is regarded by him as a transitional stage between the diffuse nontoxic and the degenerative old colloid goiter, the so-called cardiotoxic goiter.

In early toxic nodular goiter, Hertzler has found the epithelial hyperplasia not impressive. In some goiters there are newly formed acini showing a taller epithelium. In others the hypertrophy and hyperplasia are found in all acini. Nodular goiter on which exophthalmic goiter has become implanted shows the characteristic papillations. The last stage in the development of nodular colloid goiter is, in Hertzler's opinion, the cardiotoxic goiter. There is no definite cellular abnormality in this type. Changes in the colloid are the only constant feature, according to Hertzler. The colloid stains orange with Mallory's aniline blue–gold orange stain and blue with hematoxylin-eosin. This basophilic colloid, which is, since long time, known to occur in normal senile thyroids, is for Hertzler pathognomonic of the cardiotoxic goiter. He expressed the belief that the basophilic colloid enters the circulation and produces toxic effects on the heart in spite of the fact that in acini which contain this bluish or orange colloid the epithelium is wholly degenerated and "without function." Hertzler described the cardiotoxic goiter in the following dramatic paragraph:

> The cellular degenerations are most marked in those who die in a final crescendo of wild heart, delirium and high fever. The cells seem degenerated. The nuclei are desintegrated or become contracted and stain an intense color although giving a final wave of the hand to a cruel world.

Another questionable type of goiter is the "interstitial goiter" of Hertzler. The patient shows clinically the presence of a small uniform firm elastic goiter, general nervous irritability, a moderately rapid pulse, a slight loss in weight and in most cases menstrual disturbances. The size of the thyroid is about normal; the consistency is firm. The fibrous septums are prominent. It is an undeveloped, infantile gland, or it may have the gross appearance of a senile gland, according to Hertzler. The cells of the larger acini are flat or low cuboidal. The interfollicular tissue is filled with cells. These are regarded as persistent interstitial cells of infancy. Even in cases in which the interstitial cells are not conspicuous, there are noteworthy colloid changes. They are the most constant feature of the interstitial goiter. Many glands show basophilic changes of the colloid, which stains blue with hematoxylin. This indicates, according to Hertzler, an inactive colloid, which is in harmony with the flat epithelium. As treatment for the last two forms of goiter, Hertzler advises total thyroidectomy.

RELATIONSHIP BETWEEN DIFFUSE COLLOID GOITER AND EXOPHTHALMIC GOITER

It is generally recognized that following iodine medication exophthalmic goiter may resemble, at least in part, diffuse colloid goiter. It is also conceded that "secondary" exophthalmic goiter may develop in a diffuse colloid goiter. However, a functional relationship between these

two types is denied by most students of the disease. Many writers believe that colloid goiter is associated with low thyroid function and predisposes to myxedema and cretinism (Rienhoff; Breitner; Sloan).

My own studies have convinced me not only that there is an anatomic and functional relationship between diffuse colloid goiter and exophthalmic goiter but also that both occur in the same regions.

In 1927 McCarrison stated that there is little precise knowledge as to the geographic distribution and epidemiologic aspects of the diffuse colloid goiter, less as to its causes and less still as to its biochemical nature. The thesis of Marine that colloid goiter is only an involution of hyperplastic goiter has apparently paralyzed any initiative on the part of clinicians and pathologists toward study of this form of goiter. Experimental pathologists have entirely neglected it, and the lack of interest shown by clinicians is in striking contrast to the fact that most of the surgical goiters in North America develop in this structure.

The most common type of endemic goiter encountered in my autopsy and surgical material is the colloid goiter. In Aschoff's classification the colloid goiter without epithelial proliferation is distinguished from that with epithelial proliferation. The transitional stages which one observes between both suggest that they are not separate entities but different grades of the same functional stage of goiter. Also, in the so-called resting form of colloid goiter one often finds buds of epithelial proliferation in the walls of some follicles. The cells are higher in these cushion-like elevations of the epithelial wall, and new formation of small daughter acini is evident. In colloid goiter with epithelial proliferation these elevations are more extensive and much more frequent. The presence of this epithelial activity in colloid goiter, which was first described in 1920, in my paper "The Diffuse Colloid Goiter," is the strongest morphologic argument against the conception that colloid goiter is the result of involution. While Marine expressed the belief that colloid goiter never develops from a normal gland but is only the involutionary stage of hyperplastic goiter, my own studies have demonstrated that colloid goiter as observed in endemic and sporadic goiter of the lowlands develops from the normal thyroid directly.

In regard to the function of diffuse colloid goiter, most writers contend that it is hypoactive (Rienhoff; Plummer). I examined many nurses and college girls in Wichita, with and without enlarged thyroids, and I found that most of the girls with colloid goiter had a normal or slightly elevated basal metabolic rate. Signs of hypothyroidism were entirely missing.

In another period of physiologic hyperactivity of the thyroid, namely, during pregnancy, diffuse colloid goiter is frequently found. It seems to be a successful attempt of the thyroid to respond to the increased demand of the body for thyroxine. From my own clinical-pathologic studies

(1937) it is evident that the diffuse colloid goiter after the twenty-fifth year, without pregnancy, is associated with definite symptoms of hyperthyroidism in 81 per cent of the cases.

Since 1920 I have shown that from the diffuse colloid goiter transitional stages with higher and higher proliferation of the wall of the follicle may develop and lead finally to the classic picture of papillary exophthalmic goiter. For a long time before the preoperative use of iodine became general, pathologists (Kocher; MacCallum; Roussy and Clunet) pointed out that many exophthalmic goiters present on the cut surface, either throughout or in patches, an amber red translucent appearance suggesting a tissue rich in colloid and that microscopically large colloid-filled follicles are often found in otherwise typical exophthalmic goiters. I (1928) saw them in nearly 85 per cent of untreated exophthalmic goiters. Rienhoff attributed the wide, colloid-filled condition of the follicles to the preoperative administration of iodine. Careful study of histologic descriptions of exophthalmic goiter in the literature before 1923 proves the error of this conception. Without denying the striking effect of iodine on the colloid storage in hyperplastic goiter, one must not forget that evidence of a previous diffuse colloid goiter can be demonstrated in the majority of exophthalmic goiters, whether or not iodine has been given.

Clinical and histologic studies indicate, therefore, that diffuse colloid goiter and exophthalmic goiter are two related stages of thyroid activity and that one changes frequently into the other. Also, comparative geographic studies point to an association between these forms. An editorial in *The Journal of the American Medical Association* dealt recently with the comparative geography of toxic goiter. Attention was called to the discrepancy between Crotti's standpoint, based on statistics of North American goiter, and that of Sällström, who examined the geographic distribution of thyrotoxicosis in Sweden. Crotti stated that in North America exophthalmic goiter is associated with the distribution of simple goiter, while Sällström observed no geographic correlation between these two thyroid diseases. I believe that Crotti and Sällström are both correct and that the different conditions in America and Sweden are due to the different types of endemic goiter prevailing in these two regions. It is known from geographic studies that in mountainous regions where severe endemicity prevails the parenchymatous colloid-poor goiter predominates; this type of goiter is hardly ever associated with toxic symptoms. It is the anatomic basis of cretinism (Wydler; de Quervain; Wegelin). Aschoff pointed out at the first International Goiter Conference that exophthalmic goiter is independent of endemic goiter, and he was right as far as the centers of severe goiter are concerned. In level countries, on the other hand, as in North America, the prevailing endemic goiter is the colloid goiter.

The latter is closely related to the exophthalmic goiter on histologic and functional grounds; therefore, it is not surprising that thyrotoxicosis occurs in just those regions where colloid goiter is most common. One understands then why Rice found the incidence of toxic goiters in Berne to be only 3.9 per cent, as compared with an incidence of 87.5 per cent in Minnesota.

Only a complete disregard of the fundamental histologic and functional differences between endemic goiters in level regions, such as North America, Northern Germany and the Russian plains, and in mountainous centers of severe goiter, such as Switzerland and the Himalayas, explains the fear of prominent goiter surgeons (Shivers; Sloan) that cretinism also may be expected soon in this country. I feel sure that as long as colloid goiter is the predominating type of endemic goiter in North America cretinism will never become a national problem.

Since Marine's view was generally accepted that colloid goiter is not a genuine type of goiter but only an involutionary stage of hyperplastic goiter, experimental pathologists have entirely neglected it. I was the first who tried to produce this type of goiter in experimental animals. By giving a positive goitrogenic agent (calcium in excess) and ten times the normal requirement of iodine, I have many times produced typical colloid goiters in white rats. Using as a measure of thyroid function the sensitivity of the goitrous animals to lack of oxygen, I found that the colloid goiters produced by a diet rich in calcium and in iodine were much more active than the parenchymatous goiters produced by a diet rich in calcium and poor in iodine. The experimental colloid goiters resembled in structure and function the human colloid goiters of the lowlands; they were more active than normal glands.

The conception of diffuse colloid goiter as closely related to thyrotoxicosis is not only of academic interest but also of greatest practical importance. If my conception is right, that the diffuse colloid goiter forms the anatomic basis for the development of exophthalmic goiter, one may expect that successful prevention of colloid goiter will lead ultimately to the disappearance of exophthalmic goiter and hyperthyroidism. This result seems to be already in sight. From centers of goiter in this country, it is reported that since the introduction of iodized salt not only has colloid goiter in school children decreased but the number of operations for toxic goiter has been markedly reduced.

COMMENT

Histophysiologic studies of the normal thyroid form the scientific basis for evaluating the activity of the thyroid by microscopic examination. From the recent work of cytologists, physiologists and experimental pathologists the following conclusions seem justified:

The colloid represents the anatomic substratum of the active secretion of the thyroid.

The activity of the thyroid is cyclic. The first phase consists of the production and intrafollicular storage of colloid. The second phase is characterized by resorption of the colloid. Basal secretion directly into the circulation, without previous storage in the follicle, is unlikely.

Columnar epithelial cells have the function of resorption; cuboid cells are linked to colloid secretion. Cells of different height and therefore of different functional value may be found not only in different follicles of the same gland but even in the same follicle.

The colloid release can be precipitated by an injection of an extract of the anterior lobe of the pituitary containing the thyrotropic factor, a diet rich in calcium or resection of three fourths of the thyroid leading to regenerative hyperplasia. During colloid resorption the intrafollicular colloid is transformed into a thinner, more soluble state.

For normal as well as for increased function of the thyroid the maintenance of the cyclic mechanism of colloid storage and colloid release is indispensable. Preponderance of colloid release over colloid production leads necessarily to exhaustion of the gland.

In the light of these fundamental principles of the histophysiology of the thyroid it is apparent that the activity of a given thyroid cannot be judged from the colloid content alone. Breitner assumed that the colloid is produced continuously and always at the same rate. Therefore, he interpreted scarcity of colloid as anatomic evidence of increased resorption and of hyperfunction, abundance of colloid as evidence of sluggish resorption and decreased thyroid activity. He overlooked the fact that the amount of colloid depends first of all on the rate of secretion, which has never been proved to be constant. Clinical-pathologic observations do not support Breitner's hypothesis. The diffuse parenchymatous goiter and the fetal adenoma, the most common forms of goiter in cretins, and the lymphadenoid goiter are all very poor in colloid and are associated with definite symptoms of low thyroid function. On the other hand, the diffuse colloid goiter, with its abundance of colloid, is in adults often associated with hyperthyroidism. Therefore, the histologic picture of colloid storage is not identical with low thyroid activity.

The size and shape of the thyroid cells are a more reliable criterion of activity. Goormaghtigh and Thomas expressed the opinion that by counting the segments of columnar epithelium and by measuring their extent it is possible to determine almost mathematically the functional value of a given gland. The difficulty of depending entirely on these epithelial changes lies in the fact that they are present not always throughout the gland but often only in small areas. The focal nature

of the epithelial proliferations makes it necessary to examine many sections from different parts of the gland. While papillary proliferation of columnar epithelium is found almost exclusively in exophthalmic goiter, there are exceptions. Goiter in adolescents in regions of severe goiter may have all the characteristics of exophthalmic goiter and still be associated with normal or low thyroid function (Hotz; Klose and Hellwig; Stahnke; Orator). In adults papillary proliferation and columnar epithelium may be present in well encapsulated nodules without being associated with toxic symptoms.

Since typical exophthalmic goiters may be found in patients without exophthalmos, Hertzler's statement that papillary proliferation always indicates exophthalmos and that solid hyperplasia indicates absence of ocular signs is not correct. Almost all students of goiter find it impossible to diagnose the presence or absence of ocular signs from the microscopic slide. The so-called solid form of exophthalmic goiter is apparently not a special type but the end stage in the epithelial proliferation of exophthalmic goiter. Not infrequently one finds papillary and solid proliferations in the same microscopic slide. In studying the pathologic changes following Plummer's treatment, the focal nature of epithelial proliferation has to be considered. Rienhoff's view that these areas of different structure are due to hypoinvolution or hyperinvolution following treatment with iodine is based on the unfounded belief that every exophthalmic goiter is of uniform, homogeneous texture previous to administration of iodine.

The dense small areas composed of slitlike narrow acini, of pale swollen cells and of lymphoid tissue, which one finds in about one fourth of exophthalmic goiters, were described by Kocher a long time before Plummer introduced preoperative treatment with iodine. They cannot be considered, therefore, as hyperplastic areas which have not responded to iodine by involution, as Rienhoff held. Since these described areas resemble completely the histologic picture of lymphadenoid goiter, which has a low thyroid function, Kocher's stand that they are localized foci of exhaustion seems justified. The acinar cells in these dry, dense areas have lost the ability to synthesize intrafollicular colloid and do not respond even to large doses of iodine by secretion of colloid. While in exophthalmic goiter the colloid release is intensive, the synthesis of intrafollicular colloid must also be increased; otherwise the whole gland would show the same picture of exhaustion as one sees in lymphadenoid goiter.

The question as to whether toxic goiter is caused by excessive secretion of normal thyroxine, or whether there is a chemical alteration of this hormone, has not been answered. Plummer's distinction between exophthalmic goiter and toxic nodular goiter as different clinical and

pathologic entities is based on the assumption that in the first a toxic secretory product is produced, in the second an excessive amount of normal thyroxine. There is no morphologic finding to support this theory. Troell expressed the opinion that thyrotoxicosis, whether from exophthalmic goiter or from toxic nodular goiter, is characterized by the secretion of a specific staining toxic colloid. The latter appears, according to him, blue with the Heidenhain-Mallory stain, while "atoxic" colloid stains red. Mayer and Fürstenheim confirmed these findings but stated that the blue color is due only to a more liquid state of the colloid and not to a chemical alteration. The fact that one finds sometimes in the same follicle colloid which stains red in the center and blue in the periphery supports the view that the change in color is due to a difference in concentration only.

The study of the finer intracellular structures of the epithelial cells, with consideration of mitochondria, intracellular vacuoles, lipoid granules and Golgi apparatus, has also failed to reveal other than quantitative differences between the normal thyroid, simple goiter and thyrotoxicosis. The old unitarian standpoint of Möbius that there is a continuous chain of clinical symptoms ranging from the monosymptomatic "goiter heart" to hyperthyroidism and finally to the syndrome of exophthalmic goiter still holds. It is recognized that in about 20 per cent of the patients with symptoms of exophthalmic goiter exophthalmos is not present, and, on the other hand, that all symptoms, including ocular signs, may be found in those with toxic nodular goiter. "Hyperthyroidism" and "nodular toxic goiter" are not synonyms, because hyperthyroidism is associated just as often with diffuse as with nodular colloid goiter. In not more than 4 per cent of all cases of thyrotoxicosis is fetal adenoma the cause of the hyperthyroidism. Rienhoff's standpoint that true neoplasms can never become toxic is not supported by facts. There are cases reported in the literature in which the enucleation of a single adenoma was followed by prompt relief from the toxic symptoms. It is well known that in other endocrine glands (hypophysis, adrenals, pancreas) symptoms of hyperactivity may be caused by the development of true adenoma. I agree with Ginsburg, who lately reviewed the hundred year old history of toxic adenoma of the thyroid, that exophthalmic goiter and toxic nodular goiter are merely variations of a single disease and that there is no fundamental difference in nature between the two syndromes. At the Third International Goiter Conference, in 1938, Haines of the Mayo Clinic still pleaded for adherence to Plummer's division of toxic goiter into exophthalmic goiter and adenomatous goiter with hyperthyroidism. But he admitted that both conditions have many factors in common and should be classified not as separate diseases but as different clinical syndromes. With this

restriction, I believe, every pathologist can agree, provided the term "adenomatous" is omitted. It is a fact that hyperthyroidism is caused as often by diffuse as by nodular goiter. The underlying pathologic change is apparently the same in the syndrome of exophthalmic goiter as in that of hyperthyroidism, in diffuse as well as in nodular toxic goiter. As far as the morphologist is concerned, the only disturbance which can be demonstrated is epithelial proliferation associated with excessive secretion and resorption of the thyroid hormone. Not only morphologic but also chemical and biologic methods have failed to reveal an abnormal chemical constitution of the secretion in toxic goiter.

SUMMARY

Morphologic studies including special stains of the colloid and of the finer intracellular structures fail to reveal fundamental differences between exophthalmic goiter and goiter with hyperthyroidism. Both are variations of a single disease.

Epithelial proliferation and liquefied colloid are the only constant morphologic characteristics of toxic goiter.

Lymphoid tissue alone, without epithelial proliferation, is not a reliable criterion of hyperfunction of the thyroid.

The histologic structure of a diffuse goiter from a patient with the symptoms of exophthalmic goiter is typical in almost all instances. A positive clinical diagnosis can be made from the slide almost always; also, after iodine medication.

Hyperthyroidism is caused not only by nodular but as often by diffuse colloid goiter. The clinical term "adenomatous goiter with hyperthyroidism" should therefore be replaced by "hyperthyroidism."

Microscopic study of nodular goiter does not allow a histophysiologic diagnosis. Toxic and nontoxic nodular goiter are clinical, not anatomic, diagnoses.

The pathologist should use anatomic terms in describing the histologic changes observed in goiter specimens.

The universal adoption of an international classification of goiter is recommended.

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Notes and News

University News, Promotions, Resignations, Appointments, Deaths, Etc.—Richard W. Linton has been appointed assistant professor of pathology in Cornell University Medical School, New York.

George W. Corner, professor of anatomy at the University of Rochester, Rochester, N. Y., has been appointed director of the department of embryology of the Carnegie Institution of Washington, D. C., to succeed George L. Streeter on his retirement, May 1, 1940.

Medical Fellowships.—Fellowships in the medical sciences administered by the medical fellowship board of the National Research Council will be available for the year beginning July 1, 1940. These fellowships are open to citizens of the United States or of Canada who have the degree of Doctor of Philosophy or of Doctor of Medicine and are intended for recent graduates rather than for persons already established professionally. The fellows will be appointed at a meeting to be held about March 1, and applications must be filed on or before January 1. For information address Medical Fellowship Board, National Research Council, 2101 Constitution Avenue, Washington, D. C.

Directory of Medical Specialists.—During this month the Advisory Board for Medical Specialties will issue the first edition of the Directory of Medical Specialists, listing about 14,000 certified specialists. The secretary of the American Board of Pathology calls the attention of the diplomates in pathology to the value of the forthcoming directory. Dr. Paul Titus, 1015 Highland Building, Pittsburgh, is the directing editor.

Awards.—The Theobald Smith gold medal of the George Washington University was awarded, at the recent meeting of the American Academy of Tropical Medicine in Memphis, Tenn., to Richard P. Strong, of Harvard Medical School, for distinguished work in tropical diseases.

The Clement Cleveland medal of the New York City Cancer Committee has been awarded to Francis Carter Wood, director of the Institute of Cancer Research of Columbia University, in recognition of his work in connection with the committee's cancer exhibit at the World's Fair, New York.

Inter-American Society of Microbiology.—Steps have been taken toward the formation of a society of this name in order to effect a better exchange of the results of work in microbiology in Latin and North America. The establishment of an Inter-American journal of microbiology is contemplated, also an Inter-American congress on microbiology at Rio de Janeiro within the next three years. The first president of the society is Dr. A. Sordelli, director of the Department of Hygiene of Buenos Aires; Dr. F. Duran-Reynals, of Yale University, is the executive secretary.

University News, Promotions, Resignations, Appointments, Deaths, Etc.—James H. Peers has resigned as assistant professor of pathology in the University of Missouri; he will be succeeded by Henry H. Sweets Jr.

CORRECTION

In the article by Harold H. Noran entitled "Placenta Accreta," which appeared in the October issue (ARCH. PATH. **28**:532, 1939), the legends for figures 1 and 3 were reversed; in other words, figure 3 is the photomicrograph showing the normal placenta.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Pathologic Anatomy

Spontaneous Subarachnoid Hemorrhage and Congenital "Berry" Aneurysms of the Circle of Willis. H. S. Martland, Am. J. Surg. **43**:10, 1939.

Of 10,000 routine necropsies made by the medical examiner of Essex County, N. J., from 1925 to 1938, 2,500 had to do with sudden or unexplained death which necropsy revealed to have been due to natural causes. In 54 cases, or 2 per cent, spontaneous subarachnoid hemorrhage was found. Spontaneous subarachnoid hemorrhage is defined as bleeding from the arachnoid and pia mater in the absence of disease of the brain, meninges or skull. The youngest of the subjects was 14 and the oldest 67. The greatest incidence was in persons in the third, fifth and sixth decades; the cases were equally distributed between the sexes. In 38 cases the hemorrhage was due to rupture of a congenital, so-called berry aneurysm of one of the larger cerebral arteries in or near the circle of Willis. Such an aneurysm, which is usually due to a congenital defect in the arterial wall, Eppinger named "berry" aneurysm because of its shape and color. It varies in size from 3 mm. to 1 cm. It is due to lack of muscular coat at the point of sacculation and to subsequent degeneration of intimal elastica. In 5 of the 54 cases the source of bleeding could not be determined; in 9 the hemorrhage was due to a ruptured hypoplastic cerebral artery; in 1 case an arteriosclerotic aneurysm ruptured, and in the last of the series the rupture occurred in an undilated arteriosclerotic blood vessel.

I. Davidsohn.

Pulmonary Arterial Aneurysm in Tuberculous Cavities. O. Auerbach, Am. Rev. Tuberc. **39**:99, 1939.

In 1,114 cases of ulcerative pulmonary tuberculosis, 45 pulmonary arterial aneurysms were found at autopsy (34 in white persons and 11 in Negroes). The age of the patients ranged from 20 to 65 years; the greatest incidence was in the group aged 20 to 40 years. Aneurysmic dilatations were present in chronic cavities and in the left lung more frequently than the right. The size varied from 5 mm. to 3 cm. The size of the cavity bore no relation to the size of the aneurysm. Serial sections (3 cases) indicated that the genesis of such an aneurysm is as follows: (*a*) the adventitia and media of the artery are replaced by granulation tissue from the wall of the cavity as the vessel encroaches on the vomica (replacement is preceded by internal thickening in the involved region); (*b*) immediately following replacement of the adventitia and media by granulation tissue, the fibrin membrane of the inner wall of the cavity in this region extends to the intima of the vessel; (*c*) when fibrin has completely replaced the fused cavity wall and artery in the circumscribed areas, there occurs a bulge of this portion of the vessel into the lumen of the cavity; (*d*) the bulge of the vessel becomes progressively larger until it causes a tear in the thinnest portion of the wall. Aneurysms of the pulmonary artery develop in chronic cavities in which extensive healing changes have taken place. The progressive healing in the cavity brings about an increase of granulation tissue, which encroaches on a pulmonary artery in a circumscribed area and results in destruction of the elastic fibers. This weakened area becomes the site of an aneurysmic dilatation. Twenty-eight (62 per cent) of the 45 patients dying of rupture of an aneurysm of the pulmonary artery

gave a history of chronic pulmonary tuberculosis, present for from two to nineteen years, which supports the conclusion that aneurysms of the pulmonary artery tend to develop in the more chronic forms of pulmonary tuberculosis.

H. J. CORPER.

A STUDY OF THE RELATION BETWEEN NEPHRITIS AND NEPHROSIS. C. G. PANTIN, Guy's Hosp. Rep. **88**:456, 1938.

The number of hyaline glomeruli and the average diameter of the glomerular tufts were studied in normal kideys and in 21 that showed glomerulitis. It was found that by the time this disease has lasted a year completely hyaline glomeruli are usually present in some number and that the glomerular tufts are markedly enlarged, apparently by the deposition of hyaline material in them. The same and additional features were investigated in 5 cases of genuine nephrosis; it was found that the glomeruli could not be distinguished from the normal. Seventeen cases of the nephrotic syndrome in which the disease began with insidiously spreading edema are presented as a series linking cases of genuine nephrosis with cases in which obvious blood was present in the urine. Fibroepithelial "crescents" in the glomerular capsular space are derived from an organization of blood shed from the glomerulus.

FROM AUTHOR'S SUMMARY.

CHRONIC ARTHRITIS IN WILD MAMMALS. HERBERT FOX, Tr. Am. Philos. Soc. (pt. 2) **31**:73, 1939.

An analysis of observations on more than 1,700 skeletons and carcasses of wild animals indicates that in the joints changes occur which correspond with chronic arthritis in man. This has been discovered not only in specimens exhibited in menageries but also in material that was certainly in its proper wild habitat when killed. The lesions of these truly wild animals are entirely comparable to those in captive specimens. It is evident, therefore, that chronic arthritis occurs in nature. It has not been difficult to discover these changes, and it has been reasonably simple to learn which varieties of animals have the most conspicuous lesions, namely, anthropoid apes and baboons, Felidae, Hyaenidae, Ursidae, Bovidae, Cervidae and a few others. Reversely it has developed that a number of groups, notably certain families of Carnivora (e. g., Canidae) and some orders (e. g., Rodentia and Chiroptera), do not appear to have arthritis as far as the material of this study goes, although a very considerable number have been examined. The ease of discovery of the disease in hyenas and gorillas should be emphasized. No attempt is made to give percentages; it is stated only that of 1,749 skeletons 77 were accepted as arthritic. Arthritis is seen best as an involvement of the spinal column, but it occurs also very extensively in the appendicular skeleton. The distribution of lesions in the different kinds of animals suggests that there may be a relationship of function and localization of disease, the lesions being possibly related to locomotion and the jolt shock associated therewith. Conspicuous arthritis-bearers are macrosomic animals; small bodied animals, rodents and bats, etc., are missing. There is no apparent relationshp between arthritis and taxonomic position, zoogeographic or ecologic status, habits, diet, pathologic panels and focal infection. There is a strongly suggested similarity between the arthritis of the lower mammals and the deforming and rheumatoid arthritides in man.

FROM AUTHOR'S ABSTRACT.

TRAUMATIC ORIGIN OF ANEURYSMS IN ARTERIES AT THE BASE OF THE BRAIN. G. KAHLAU, Frankfurt. Ztschr. f. Path. **51**:319, 1938.

Kahlau discusses the relationship between trauma to the skull and formation of aneurysms. Fourteen incidences are cited from the literature in which aneurysms were supposedly due to trauma affecting the skull without any evidence of injury. Two cases are described. In the first case the trauma had caused an irritation

of the arteries at the base of the brain (contrecoup). As a result, the circulation in the vasa vasorum within the walls of the arteries was disturbed. Owing to insufficient nutrition of the walls, necrosis followed. Stasis in the vasa vasorum may also have occurred, with subsequent erythropedesis. In this way Kahlau explains the presence of pigment in the adventitia. The necrotic area in the wall may have torn and been repaired by granulation tissue. Distinct signs of these changes were present in the first of the author's cases. In addition, small scars were found in the walls of the arteries which may have been stretched by the blood pressure, causing the formation of aneurysms. In the second case, aside from the aneurysm there were no scars present in the involved artery. The wall of the aneurysm, however, consisted of connective tissue. Since no etiologic factor other than trauma could be elicited, the author believes that this aneurysm was of traumatic origin. In supporting this point of view he stresses the localization of the aneurysm at the point where contrecoup had occurred.

OTTO SAPHIR.

FAT EMBOLI IN EXPERIMENTAL SODIUM OR AMMONIUM HYDROXIDE POISONING. I. G. FAZEKAS, Frankfurt. Ztschr. f. Path. **51**:524, 1938.

Previous studies by Fazekas revealed that poisoning with ammonium hydroxide may cause severe fat embolism. In this poisoning and in that by sodium hydroxide, hyperglycemia, hyperphosphatemia, hypocalcemia and severe acidosis occur. Fazekas investigated the presence of fat emboli in various organs of experimental animals poisoned with sodium hydroxide and emphasized some work on fat embolism in experimental ammonium hydroxide poisoning. He points out that fat emboli were found in 94 per cent of the animals poisoned with sodium hydroxide and in 97 per cent of the animals poisoned with ammonium hydroxide. Fat emboli occurred in the lungs in 80 to 90 per cent of the animals, in the choroid plexus in 57 per cent and in the kidneys in 20 per cent. Very rarely were they present in liver, spleen or heart (5 to 10 per cent). The fat embolism in these poisonings when occurring in the lungs and brain may be so severe as to cause death. Sudden death following these poisonings, which formerly was attributed to shock, may be thus explained. The fat emboli were due to lipemia, which appeared as a result of the poisoning with both sodium hydroxide and ammonium hydroxide.

OTTO SAPHIR.

ANEURYSMS AT THE BASE OF THE BRAIN. L. E. C. SCHMIDT, Frankfurt. Ztschr. f. Path. **51**:539, 1938.

Schmidt, in presenting 8 cases of aneurysm at the base of the brain, attempts to explain the development of such an abnormality. In all 8 cases the following observations were made: The location of the aneurysm was at a sharp angle formed by the branching of vessels. The musculature of the media stopped abruptly at the point where the vessel passed into the aneurysm. The internal elastic lamella was split and fragmented within the aneurysmal wall, which consisted of dense connective tissue with some elastic fibers. The adventitia was loose and revealed granulation tissue, iron-laden cells and red blood cells. In the ruptured aneurysm the wall at the site of rupture was thinned and contained fibrin. It is stressed that arteriosclerotic changes may also weaken the wall. Evidence of syphilis was not found. Eleven control cases without aneurysms revealed on routine examination interruptions of the media of three different types: (1) scars which were caused by inflammatory processes of a specific or a nonspecific nature; (2) defects in the media beneath sclerotic areas of the intima, apparently caused by stretching of the muscularis or by disturbances of nutrition, and (3) defects at points of branching vessels. The intima in these regions was slightly thickened and the muscularis of the media interrupted as in the cases of aneurysm. These defects seemingly occur during embryologic development by the formation and involution of branching vessels. Thus, the aneurysm is located

either at the sharp angle of a bifurcation or at a defect in the wall of the vessel, the site of an involuted branch. The similarities between the structure of the wall of the aneurysm and the defects in the media in control cases, the formation of multiple aneurysms in a single case and the absence of inflammatory infiltration in the aneurysm itself are given as evidence that the defects normally found in the media bear a causal relation to the formation of aneurysms.

OTTO SAPHIR.

Microbiology and Parasitology

GROWTH OF THE FOWL CORYZA BODIES IN TISSUE CULTURE AND IN BLOOD AGAR. J. B. NELSON, J. Exper. Med. **69**:199, 1939.

Evidence is presented that the capacity of chick embryo tissue to promote growth of the fowl coryza bodies is conditioned by a diffusible cellular component which is essential for the multiplication of these bodies. This growth factor is inactivated at p_H 6 but withstands a temperature of 100 C. for sixty minutes. An amount sufficient to promote normal growth of the specific bodies may be present in tissue culture supernatants long after the content in the tissue is exhausted. Postembryonic tissue (liver and spleen) contains a variable amount of growth factor and is not a satisfactory substitute for the chick embryo. Multiplication of recently isolated fowl coryza bodies is not demonstrable in nutrient mediums enriched with blood. Experiments with one strain, however, indicate that an adaptation to fluid blood in an agar medium may take place after many generations in tissue culture.

FROM AUTHOR'S SUMMARY.

VIRUS DISEASE OF CATS. W. D. HAMMON and J. F. ENDERS, J. Exper. Med. **69**:327, 1939.

An acute, highly fatal epizootic disease of cats is described, which can be recognized by: extreme leukopenia of a fulminating type, involving all types of white blood cells; aplasia of the bone marrow, including both the granulocytic and the erythrocytic series and occasionally the megakaryocytes; aplasia of lymphoid tissue, and characteristic intranuclear inclusion bodies in the cells of the intestinal mucosa and in certain cells of the spleen, lymph nodes and bone marrow. The infection has been induced in healthy cats by means of bacteria-free filtrates of emulsions of the spleens of infected animals. Collateral evidence supports the conclusion that the disease is due to a virus. The pathogenicity of the infectious agent has proved thus far to be strictly limited to the natural host.

FROM AUTHORS' SUMMARY.

VITAMIN C IN RELATION TO POLIOMYELITIS. A. B. SABIN, J. Exper. Med. **69**:507, 1939.

In the experiments reported in the present communication it was found that vitamin C, both natural and synthetic preparations, had no effect on the course of experimental poliomyelitis induced by nasal instillation of the virus. The objection cannot be raised that too large an amount of virus was used, since recent studies on the fate of nasally instilled virus indicated that all but an undetectable amount of it is swallowed and disappears from the nasal mucosa within three hours or less and that none is demonstrable in the central nervous system before the third day. The administration of vitamin C was begun immediately after the instillation of virus, and if it were capable of exerting any effect on the virus or the tissues it could have done so even before multiplication of virus had begun. Monkeys whose store of vitamin C was depleted reacted in the same way as those receiving an adequate diet. There is no apparent explanation for the difference between these results and those reported earlier by Jungeblut. During the present investigation it was found that monkeys on a scorbutic diet died of spontaneous acute infections, chiefly pneumonia and enterocolitis, while their mates receiving

an adequate diet remained well. The surviving monkeys on the scorbutic diet showed the osseous and other changes of human scurvy, and the vitamin C used in this study was shown to produce healing and calcification in the bones as well as to check the edema and hemorrhagic diathesis.

FROM AUTHOR'S SUMMARY.

THE BLOOD STREAM IN EXPERIMENTAL POLIOMYELITIS. F. B. GORDON and E. H. LENNETTE, J. Infect. Dis. **64**:97, 1939.

Multiple transfusions of blood from infected monkeys were made with simultaneous damage to the blood–central nervous system barrier of the recipient monkeys, in an attempt to demonstrate for poliomyelitis virus in the blood stream. The recipient monkeys were subsequently tested for immunity.

These methods did not demonstrate the virus in the blood, 14 samples of which, ranging in volume from 10 cc. to 45 cc. were taken from paralyzed monkeys and 28 samples, 8 cc. to 30 cc. in volume, from infected monkeys before paralysis appeared.

Repeated intranasal inoculation of 7 monkeys after destruction of their olfactory bulbs did not result in a clinical attack of poliomyelitis, in the appearance of virus in the blood or in a detectable immunologic response. No evidence, therefore, was found for the absorption of virus by the blood stream or by other routes from nasal or alimentary mucosa.

The results indicate that the virus of poliomyelitis either does not enter the blood stream of intranasally inoculated monkeys or does so in amounts too small to be detected by the methods employed. FROM AUTHORS' SUMMARY.

CULTIVATION OF VACCINIA VIRUS IN DEEP COLUMNS OF MAITLAND MEDIUM. R. L. THOMPSON and M. S. COATES, J. Infect. Dis. **64**:105, 1939.

"Multiplication of vaccinia virus was obtained in columns of Maitland medium up to 115 mm. in depth. It is apparently essential that a suitable tissue-fluid ratio be observed in order to obtain reasonable growth of virus.

"Increasing the oxygen tension in cultures by passing air through the medium did not enhance growth of the virus.

"Decreasing the oxygen tension in cultures by excluding the air inhibited growth of the virus. Rough determinations indicate that the oxidation-reduction potential in such cultures is about + 150 millivolts.

"The New York City Board of Health strain of vaccinia virus was carried through 25 serial transfers in columns of Maitland medium, having an average depth of over 45 mm., without change of virulence."

FROM AUTHORS' SUMMARY.

INFECTION IN THE MOUSE FOLLOWING INTRATRACHEAL INOCULATION OF AN ATYPICAL PERTUSSIS ORGANISM. W. L. BRADFORD and M. WOLD, J. Infect. Dis. **64**:118, 1939.

With intratracheal or intranasal inoculation of a recently described organism resembling Haemophilus pertussis a definite pulmonary lesion is produced in mice. This lesion is of the order of interstitial pneumonia and essentially resembles that produced by inoculation with H. pertussis.

FROM AUTHORS' SUMMARY.

THE NUTRITIVE REQUIREMENTS OF THE SALMONELLAS. W. BURROWS, J. Infect. Dis. **64**:145, 1939.

Evidence is presented which indicates that tryptophan is not essential to the growth of those strains of the typhoid bacillus which apparently require it. The amino acid exerts a growth-stimulating effect similar to sodium sulfide in glucose-

ammonia solutions. The ability of these organisms to synthesize tryptophan is shown by analysis of cultures before and after growth. The typhoid bacillus, contrary to usual belief, produces indole in extremely small amounts.

FROM AUTHOR'S SUMMARY.

CHEMICAL CHANGE DURING LYSIS OF B. COLI. A. PIRIE, Brit. J. Exper. Path. **20**:99, 1939.

Lysozyme is a principle contained in various animal fluids which is capable of dissolving certain bacteria. It is now recognized that lysozyme preparations contain a carbohydrate-splitting ferment. Pirie finds that during lysis of Bacterium coli by its phage a similar carbohydrate-splitting enzyme is active, but that the enzyme is not present in concentrated phage preparations. She concludes that growth either of phage or of bacteria is unnecessary for lysis to occur, but that the disintegration may be brought about by the bacterial enzymes.

METASTATIC LESIONS FROM TUBERCLE BACILLI IN PARAFFIN. N. RIST, Ann. Inst. Pasteur **61**:121, 1938.

A suspension of dead tubercle bacilli in saline solution produced lesions only at the site of injection, but a suspension in paraffin or paraffin oil was found to have caused lesions in adjacent lymph nodes and lungs. The conditions of actual infection were more closely approximated, with the production of epithelioid nodules surrounded by lymphocytes and fibrosis. In the center appeared the nidus of organisms, often surrounded by polymorphonuclear cells and a necrotic area. Paraffin oil itself tends to migrate, without producing lesions, and the organisms appear to metastasize with its support. There appeared to be protection against phagocytosis. A large purulent lesion was formed within a year at the site of inoculation.

FROM AUTHOR'S SUMMARY.

LABORATORY INFECTION WITH LYMPHOCYTIC CHORIOMENINGITIS. P. LÉPINE and V. SAUTTER, Ann. Inst. Pasteur **61**:519, 1938.

A strain of the virus of lymphocytic choriomeningitis isolated in Paris was similar to strains recovered in the United States. The disease began with a period of symptoms resembling those of influenza, followed by a free period and then by a period in which the number of cells in the spinal fluid was high. Lack of evident contagiousness rendered previous data equivocal, indicating the significance of a laboratory infection in one of the authors (V. S.). The disease in this case lasted twenty-two days, with a maximum temperature, early and again about the twelfth day, of 39.2 C. (102.5 F.). Virus was recovered from both blood and urine. Serologic tests of others working with the virus indicated no latent infections. It is probable that infection occurred when a particle of glass flew into the conjunctiva of the victim while she was grinding an antigen of lymphocytic choriomeningitis in a mortar ten days before symptoms appeared.

M. S. MARSHALL.

INNOCUOUSNESS FOR MAN OF THE RICKETTSIA OF THE TYPE R. ROCHA LIMAE, P. DURAND and H. SPARROW, Arch. Inst. Pasteur de Tunis **28**:14, 1939.

Conclusions are drawn from three years of experience in Tunis and in France, the latter surely not in a region where lice confused the picture. Intraperitoneal, subcutaneous and conjunctival inoculation produced neither local nor general reactions in monkeys, rabbits, guinea pigs and rats. Neither intramuscular nor conjunctival inoculations of man resulted in infection, nor did persons hired to permit feeding on their skins of large numbers of infected lice show infection. The rickettsias did not appear even to survive in man, for the persons who nourished infected lice for twelve days and then fed uninfected lice for forty-six days did not infect the latter.

FROM AUTHORS' SUMMARY.

Immunology

EFFECTS OF ANAPHYLAXIS AND HISTAMINE ON THE HEART. E. C. ANDRUS and H. B. WILCOX JR., J. Exper. Med. **69**:545, 1939.

Anaphylaxis in isolated, perfused hearts of cats has been shown to be accompanied by considerable, though transient, increase in coronary flow. This result is contrasted with that observed in the hearts of guinea pigs and rabbits, in which the coronary arteries are constricted during anaphylaxis. Attention is directed to the fact that in the hearts of these three species (guinea pigs, rabbits and cats) the effects of anaphylaxis and of histamine are qualitatively parallel. The characteristic anaphylactic response in the isolated hearts of guinea pigs has been evoked: (*a*) in hearts removed from immune animals, (*b*) by each of two antigens (horse serum and egg albumin) under conditions of double sensitization and (*c*) on exposure of the hearts of passively sensitized animals to the type-specific polysaccharide of the pneumococcus. It is evident that among the effects of anaphylaxis on smooth muscle in various organs there must be considered that on the coronary arteries.

FROM AUTHORS' SUMMARY.

MECHANISM OF IMMUNITY IN TUBERCUOSIS. M. B. LURIE, J. Exper. Med. **69**: 555 and 679, 1939.

In a previous study (*J. Exper. Med.* **63**:923, 1936) it was found that tubercle bacilli multiplied unhindered in acellular agar islands in normal animals, while in immune animals a marked inhibition of growth was evident, apparently due to saturation of the agar with immune body fluids. The first of the two papers reviewed here presents more definite evidence of the role of humoral bacteriostatic factors in vivo, obtained by the use of another procedure. The fate of bacilli of reinfection at the portal of entry and in metastatic foci is essentially similar in the rabbit and the guinea pig, but in the guinea pig the bacilli of reinfection are more effectively fixed at the portal of entry. Unrelated substances, such as trypan blue and agar particles, are fixed more effectively at the site of reinfection in the guinea pig than in the rabbit. Precipitins from the circulating blood accumulate in higher concentration at the site of a local nonspecific inflammation in the tuberculous guinea pig than in the tuberculous rabbit. This difference in fixing capacities is associated with difference in the extracellular character of the inflammation resulting from reinfection: (*a*) in the guinea pig, highly sensitized, the adjoining lymphatics become thrombosed and the fibrinous network is fine; (*b*) in the rabbit the lymphatics remain open and the fibrinous network is coarse. In rabbits and guinea pigs primarily infected the destruction of bacilli is most extensive at the portal of entry and less effective in the nearest metastatic foci; in remote internal organs the bacilli grow without hindrance. Cell-free body fluids of normal animals support growth of tubercle bacilli in vivo; body fluids of tuberculous animals are bacteriostatic.

The second paper represents an endeavor to elucidate the mechanism of the acceleration in the mobilization of mononuclears which characterizes the response to reinfection of the tuberculous or allergic animal. In tuberculous and vaccinated rabbits and guinea pigs mononuclear phagocytes mobilized at the site of a nonspecific inflammation with greater rapidity than in normal animals, as they do also in response to tubercle bacilli. The succession of cells that characterizes inflammation is accelerated in allergic rabbits and guinea pigs in response to nonspecific irritants. The p_H at the site of reinfection and at that of a nonspecific inflammation in immunized rabbits and guinea pigs is lower than at corresponding points in normal animals. No relation was found between the mobilization of mononuclears and the hydrogen ion concentration at the site of inflammation. The rate of mitotic and amitotic division of mononuclears in allergic animals in response to nonspecific irritants is greater than that in normal animals. The mononuclears from actively tuberculous or vaccinated guinea pigs are more active in vitro toward tubercle bacilli, carbon and staphylococci. The phagocytic capacity

of mononuclears for tubercle bacilli is less if vaccination is done with a bacillus of low virulence. An increase in the phagocytic activity of mononuclears occurs in mediums containing serums derived from normal and from tuberculous persons. More rapid mobilization of mononuclears is associated with increased physiologic activity of these cells. The significance of this enhanced activity conferred by the tuberculous process on the mesenchyme is discussed in relation to immunity and other phenomena.

H. J. Corper.

Protection Against the Typhoid Bacillus. D. W. Henderson, Brit. J. Exper. Path. **20**:1, 1939.

Henderson has studied the immunologic properties of antigens extracted from typhoid bacilli by diethylene glycol. It appears that during the process structural changes occur in the antigens which lead to a loss of function.

Antigenic Surface of Smooth Brucella Abortus and Melitensis. A. A. Miles, Brit. J. Exper. Path. **20**:63, 1939.

Miles has confirmed the hypothesis that the antigenic surface of smooth Brucella abortus and Brucella melitensis contains both A and M antigens, A being the major antigen in abortus, M that in melitensis. The ratio of major to minor antigen on the surface of the two species varies between 50:1 and 10:1. The rabbit responds to this mixture of antigens by the formation of antibodies, anti-A, anti-M and apparently anti-AM, the latter bearing on one particle receptors for both antigens.

Antigenic Preparations from Brucella Melitensis. A. A. Miles and N. W. Pirie, Brit. J. Exper. Path. **20**:83, 1939.

Miles and Pirie report part of an attempt to isolate from bacteria of the Brucella group the specific soluble substances responsible for the differences in the serologic behavior of the three species, abortus, suis and melitensis; their paper deals mainly with Brucella melitensis. In the first part the preparation of the antigen from smooth Br. melitensis and the physical properties of the antigen are described. The antigen in its native state is serologically and chemically complex; it consists of a nitrogenous substance, analogous to the specific soluble substances of many bacteria, combined with a protein-like material and a mixture of lipoids.

Allergy and Immunity from Dead Tubercle Bacilli in Vegetable Oil. E. Coulaud, Ann. Inst. Pasteur **61**:355, 1938.

Killed organisms when given to guinea pigs or rabbits subcutaneously or intramuscularly in vegetable oils confer a more rapid, more definite and more lasting state of allergy than such organisms suspended in saline solution. Organisms in paraffin or paraffin oil are still more effective with respect to conferred allergy. The same order of effectiveness was noted with regard to resistance to infection. The results following subcutaneous injection using paraffin were roughly equivalent to those following intravenous injection using olive oil. The gravity of pulmonary lesions following intravenous injections, however, offset the potential resistance of some animals.

From Author's Summary.

Allergy and Immunity in Experimental Tuberculosis. K. E. Birkhaug, Acta tuberc. Scandinav. **13**:163, 1939.

Allergic hypersensitiveness to tuberculin and acquired resistance to tuberculosis are two distinct and dissociable phenomena whose concurrent presence in tuberculous infection is merely fortuitous.

Dissemination of virulent tubercle bacilli through the lymphatic, venous and systemic circulation as well as through the viscera is significantly more delayed in the completely desensitized than in the allergic hypersensitive organism.

Inoculation of virulent bacilli induces in the allergic hypersensitive organism generalized, invasive and caseonecrotic tuberculous lesions, rich in tubercle bacilli. Similar inoculation in the desensitized organism induces circumscribed, noninvasive tubercles, containing a few tubercle bacilli.

Allergic hypersensitiveness is of no fundamental importance in acquired resistance to tuberculosis except for its unfortunate effect in local and general disturbances which permit dissemination of tubercle bacilli and their inflammatory-necrotizing tuberculoproteins.

Abolition of allergic hypersensitiveness by careful tuberculin desensitization leaves the mechanism of acquired tuberculoresistance intact and significantly protects the body against active and invasive disease.

FROM AUTHOR'S SUMMARY.

Tumors

DYES AND FOREIGN PROTEINS IN NORMAL AND MALIGNANT TISSUES. F. DURAN-REYNALS, Am. J. Cancer **35**:98, 1939.

T. 1824 and other poorly diffusible dyes when injected intravenously localize selectively in spontaneous and transplanted tumors growing in mice, rabbits and chickens. Horse, pig and chicken serums when injected intravenously localize selectively in transplanted and spontaneous tumors in mice. The localization of the dyes is effected by the stroma of the tumor, the cancer cell not being penetrated by the dyestuff. Necrosis in tumors favors localization of both serums and dyes. The findings are interpreted as indicating that newly formed capillaries of tumors are, in general, more permeable than the capillaries of any normal tissue.

FROM AUTHOR'S SUMMARY.

CHANGES PRODUCED BY ESTROGEN. L. LOEB, V. SUNTZEFF and E. L. BURNS, Am. J. Cancer **35**:159, 1939.

An estrogen may affect the stroma of various organs in two opposite directions: (1) It may, by inducing growth processes in the epithelial structures and perhaps also by an effect on the circulation, cause a diminution in the amount of fibrous-hyaline material in certain organs, and there are indications that it may exert these effects in the vagina, cervix and uterus. (2) It may, if large doses are administered over long periods of time, have the opposite effect; i. e., it may cause a marked increase in the amount of fibrous-hyaline material in the stroma. In this way it is possible, therefore, to initiate and to intensify in certain organs changes similar to those occurring in old age. An increase in the amount and density of the fibrous-hyaline substance in the stroma tends to exert pressure on functionally active and proliferating tissues and may thus cause interference with the supply of food and oxygen, which in the end in certain instances may lead to destruction of the more sensitive structures. On the other hand, it is possible that constant stimulation of the connective tissue by a foreign body such as dense hyaline, perhaps in cooperation with other factors, leads in some cases to production of sarcoma.

FROM AUTHORS' SUMMARY.

THE FUNCTIONAL ACTIVITY OF THE MAMMARY GLAND OF THE RAT IN RELATION TO MAMMARY CARCINOMA. H. J. BAGG and F. HAGOPIAN, Am. J. Cancer **35**:175, 1939.

Rapid breeding and prevention of suckling (the so-called functional test of these experiments) have made it possible to detect female rats of the Wistar strain whose constitution is favorable to the formation of mammary neoplasms. Fifty-six

females were tested. With a correction for those animals that died under the cancer age, the incidence of spontaneous mammary adenocarcinoma under the conditions of the experiment was 4 in 29, or about 14 per cent. Under similar conditions fibroadenoma and adenoma of the mammary gland occurred spontaneously in 27 and 20 per cent, respectively. Adenoma of the pituitary gland, a hypertrophic cystic condition of the adrenals and leiomyoma of the uterus were noted in female rats that acquired mammary adenocarcinoma under the conditions of the experiment. Similar pathologic disturbances occurred in association with fibroadenoma or adenoma of the mammary gland. Stasis of secretions within the ducts as a result of nonsuckling of the young was a prominent feature associated with the tumors of the mammary glands. In addition to the internal factors of a hormonal nature, it is possible that the chemical irritation of the retained secretions bears a causative relation to the onset of tumors of the mammary glands in the female rat. Leiomyoma of the uterus appeared in 5 females subjected to reproductive overwork. The tumor was transplanted successfully. Fibroadenoma and adenoma of the mammary gland also grew well on transplantation. Adenocarcinoma failed to grow, and none of the original or of the transplanted growths was observed to metastasize.

FROM AUTHORS' SUMMARY.

CARCINOGENIC AND RELATED NON-CARCINOGENIC HYDROCARBONS IN TISSUE CULTURE. E. M. H. CREECH, Am. J. Cancer **35**:191, 1939.

When added to mouse fibroblasts in tissue cultures, 1,2,5,6-dibenzanthracene choleic acid caused an increase in the proliferation of these cells as compared with controls, which included cultures treated with phenanthrene choleic acid, acenaphthene choleic acid or desoxycholic acid and untreated cultures. Desoxycholic acid and phenanthrene choleic acid were found to give a decrease in cell proliferation. The increase in outgrowth of cultures containing 1,2,5,6-dibenzanthracene choleic acid over the untreated controls was approximately 50 per cent. The chromosomes showed a precocious splitting in the prophase in the cultures treated with 1,2,5,6-dibenzanthracene choleic acid, 20-methylcholanthrene choleic acid on methylcholanthrene in serum but not in any of the four types of controls. Similarities to miosis (reduction division) were occasionally found in cultures treated with 1,2,5,6-dibenzanthracene or with methylcholanthrene choleic acid, in which the chromosomes of the metaphase were lying closely in pairs, the members of which separated later to opposite poles, resulting in a reduction division and in the "node and loop" formation of the chromosomes.

FROM AUTHOR'S SUMMARY.

CHEMOANTIGENS AND CARCINOGENESIS. W. R. FRANKS and H. J. CREECH, Am. J. Cancer **35**:203, 1939.

Immunization of stock white mice with dibenzanthranylcarbamidocasein as antigen gives evidence of reducing their susceptibility to carcinogenesis from injected dibenzanthracene as compared with untreated controls.

FROM AUTHORS' SUMMARY.

EFFECT OF DIET ON TUMORS INDUCED BY ULTRAVIOLET LIGHT. C. A. BAUMANN and H. P. RUSCH, Am. J. Cancer **35**:213, 1939.

By means of ultraviolet rays tumors were produced in albino mice kept on five standard diets. The rate of tumor production varied with the diets. Tumors were produced most rapidly on a diet high in fat, whereas brain extract or liver retarded the production of tumors. Addition of 2 per cent cholesterol to the stock diet did not affect the rate, although a marked increase in hepatic fat and cholesterol resulted. No relation appeared to exist between the cholesterol content of the blood, liver, kidney, ears or skin and the production of tumors on the various diets. The results suggest that the role of cholesterol in the production of tumors

is a limited one. The cholesterol content of the rat's skin was increased by irradiation but not that of the skin of the mouse or the guinea pig. The increase appeared to be due to systemic changes. FROM AUTHORS' SUMMARY.

SARCOMA OF SOFT TISSUES. E. M. BURKE, Am. J. Cancer **35**:234, 1939.

In a series of 201 cases of soft tissue sarcoma there was a wide variation in the location of the primary lesion. The patients represented every decade of life but especially the fifth and sixth decades. Recurrences are common, and the tumor is prone to metastasize, especially in its later stages. Sarcoma of the soft tissue can readily be classified into definite histologic grades. The prognosis for the higher grades is poor, and only a small percentage of the patients remain well for a five year period. FROM AUTHOR'S SUMMARY.

A MALIGNANT TUMOR OF THE THYMUS IN A RABBIT. J. W. ORR, Am. J. Cancer **35**:269, 1939.

A malignant neoplasm of the thymus occurred in a rabbit not less than 4 years old. Metastases were found in the heart, lungs and two abdominal nodes. Reasons are given for regarding the tumor as a carcinoma of the reticulum cells.

FROM AUTHOR'S SUMMARY.

TUMOR NOMENCLATURE: SUGGESTIONS FOR ITS REVISION. H. E. ROBERTSON, Am. J. Clin. Path. **9**:24, 1939.

The present nomenclature of tumors is in serious need of critical review. The terms "benign" and "malignant" might be dropped. The implications carried by the words "benign tumor" are illogical and all "oma" suffixes for these tissue masses should be substituted, when possible, by more appropriate designations. There is no good reason to retain the names "myxoma," "endothelioma" and "epithelioma." The tumor called basal cell carcinoma is probably incorrectly labeled. Further simplification and elimination of unsuitable terms in tumor classification should be attempted. FROM AUTHOR'S SUMMARY.

COMPARISON OF VIRUS-INDUCED TUMORS WITH TAR TUMORS. P. ROUS and J. G. KIDD, J. Exper. Med. **69**:399, 1939.

Tarring the ears of rabbits of one sort with a single kind of tar evoked epidermal tumors of a few sharply defined types, namely, ordinary papillomas, carcinoids, carcinomas and "frill horns." The last, relatively infrequent, are now recognized for the first time. The carcinoids proved to be the expression of a spurious malignancy of papillomas, resulting from intercurrent influences, and they were wholly dependent on these for their threatening aspect and behavior. Chief among such influences was continued tarring. It had the effect of establishing the papillomas; it stimulated their proliferation, complicated their morphologic character and rendered some of them disorderly, aggressive and anaplastic. It brought all the tissues of the ears into an excitable state, and often this state endured long after the skin had apparently become normal. The characters of the papilloma-carcinoids and of the "frill horns" were so different and distinctive as to imply the action of differing, specific causes. The papillomas were very like those induced with the Shope virus, and hence a point to point comparison was made of their manifestations, including the derivation of carcinomas from them. This comparison demonstrated that the unknown cause of the tar papillomas provoked neoplastic phenomena which were identical in all essential respects with those due to the virus. To suppose, for experimental purposes, that the papillomas which tarring elicits are caused by a virus rendered pathogenic by this procedure is to demand least of the unknown. Yet it does not follow that they must be due to a virus.

FROM AUTHORS' SUMMARY.

UTERINE ADENOMA IN THE RABBIT. H. S. N. GREENE, J. Exper. Med. **69**:447, 1939.

The behavior of a transplanted adenocarcinoma of the uterus of a rabbit has been studied through twelve serial generations in the anterior chamber of the eye and through six serial generations in the testicle. The transplanted tumor is characterized by slow growth, which is at first expansive and later invasive, by an ability to form more or less differentiated structures in response to different environmental conditions and by late metastasis. The endocrinologic changes that distinguish animals bearing the spontaneous tumor do not occur in animals bearing the transplanted tumor. Various experiments were undertaken in an attempt to discover the nature of the factors determining the characteristics of the spontaneous and those of the transplanted tumor. It was found that successful transplantation was followed by a phase during which the host animals were refractory to reinoculation. The results of transplantation into the eyes of animals with spontaneous tumors suggested the existence of a similar phase during the early development of the tumor, but the number of observations was not sufficiently numerous to warrant definite conclusions.

FROM AUTHOR'S SUMMARY.

EFFECT OF ESTROGENIC HORMONE AND OVARIECTOMY ON THE NORMAL ANTIBODY CONTENT OF THE SERUM OF MATURE RABBITS. L. WEINSTEIN, Yale J. Biol. & Med. **11**:169, 1939.

Estrogenic substance administered to mature male and female rabbits produces an increase in the amount of circulating agglutinin for Escherichia coli and in that of hemolysin for sheep erythrocytes, the degree of change being directly related to the amount of the substance administered. Small doses of the estrogen, while capable of producing an increase in the concentration of hemolysin, are ineffective in provoking any change in agglutinin. Ovariectomy in mature rabbits is followed by a decrease and subsequently by an increase to high levels in the agglutinin content of the serum, the hemolysin rising to levels much higher than those present previous to operation. The action of ovariectomy in producing changes in the antibody content of the serum of rabbits may be related to gonadotropic hormone. The mechanism of the change produced by the administration of estrogen or by the removal of the ovaries cannot be explained at present, although there seems to be some evidence that it is not the result of infection of the uterus.

FROM AUTHOR'S CONCLUSIONS.

THE SYNERGISTIC EFFECT OF HEPTYL ALDEHYDE AND METHYL SALICYLATE ON SPONTANEOUS TUMORS OF THE MAMMARY GLAND IN MICE. L. C. STRONG, Yale J. Biol. & Med. **11**:207, 1939.

A combination of 3 parts of heptylaldehyde and 1 part of synthetic methylsalicylate added to the diet of tumor-bearing mice is an effective means of influencing malignant neoplasms by chemotherapy. Of 45 tumor-bearing mice, 20 (44.4 per cent) showed pronounced diminution in the size of their tumors following this experimental treatment. In 12 of these (26.6 per cent) the original tumors disappeared. Of these 12 mice, 9, following the regression of their tumors, lived in normal health without any evidence of recurrence. The other 3 mice (of the 12 referred to) later, when placed on the control diet, had recurrences at the sites of the original tumors. The average growth rate of all the tumors in the experimental animals was significantly lower than the corresponding growth rate for the control mice. Also, the experimental mice lived longer than the controls. The chemical significance of the use of heptylaldehyde in the treatment of spontaneous tumors in mice is discussed.

FROM AUTHOR'S SUMMARY.

FILTERABLE TUMORS IN FOWLS INDUCED BY TAR. J. McINTOSH and F. R. SELBIE, Brit. J. Exper. Path. **20**:49, 1939.

Previously McIntosh had shown that cell free filtrates from Rous tumors that had been induced in fowls by tar painting were capable of inducing tumors in fowls in series. This result suggested the possibility that a virus was the active agent in the production of these tumors. Subsequently other workers endeavored unsuccessfully to pass chemically induced tumors with cell free filtrates. McIntosh and Selbie have now repeated and extended the work, with a result similar to that of McIntosh. They give evidence in support of their view that a virus has a causal connection with these tumors.

GRANULOSA CELL TUMOR OF THE OVARY. W. F. HARVEY, E. K. DAWSON and J. R. M. INNES, Edinburgh M. J. **46**:256, 1939.

The granulosa cell tumor has its origin in primitive ovarian follicle cells. It may vary in histologic appearance, in some instances differentiating to organoid architecture and in others remaining diffuse. It is characterized by estrogenic activity. It is one of a definite group of gonadal tissue tumors and may even present in part some of the features of one or another of the chief members of the group, the arrhenoblastoma and the dysgerminoma.

FROM AUTHORS' SUMMARY.

MULTIPLE UTERINE AND EXTRAGENITAL TUMORS PRODUCED BY ESTRADIOL BENZOATE. A. LIPSCHÜTZ and R. IGLESIAS, Compt. rend. Soc. de biol. **129**: 519, 1938.

Castrated female guinea pigs receiving subcutaneously estradiol benzoate to a total of 1 to 4 mg. in four months showed many fibroma-like tumors developing on the uterus, intestine, spleen, liver and other organs. R. J. LEBOWICH.

CANCER-PRODUCING TAR FROM TOBACCO. A. H. ROFFO, Bol. Inst. de med. exper. para el estud. y trat. d. cáncer **15**:349, 1938.

Experiments were made on rabbits with tars from the smoke of nine varieties of commercial tobacco. These tars did not contain any nicotine. All the tars proved to be cancerigenic on application every second day for several months to the inside of the rabbit ear, some more so than others. Fluorescence, the findings on spectrometric examination and the effects on rabbits indicate that tobacco tar contains the same hydrocarbons as coal tar. Roffo believes that his results point directly to restriction in the use of tobacco as a means of prevention of certain forms of cancer.

AUTHOR'S ABSTRACT.

CANCER IN THE DIGESTIVE TRACT FROM THE INGESTION OF FAT OXIDIZED BY BY HEAT. A. H. ROFFO, Bol. Inst. de med. exper. para el estud. y trat. d. cáncer **15**:539, 1938.

The ingestion of fats oxidized by heating and added to the food of white rats induces malignant growths in the stomach and other organs, but slowly, usually after about two years. The lesions in the stomach are round ulcers with papillomatous borders in which adenocarcinoma develops. In the liver spindle cell sarcoma may arise. The cancerigenic effects of such fats is ascribed to the formation of oxycholesterol. The results are similar to those obtained previously by Roffo from cholesterol oxidized by ultraviolet radiation.

FROM AUTHOR'S SUMMARY

GEMMANGIOMA AND ITS RELATIONSHIP TO ANGIOSARCOMA. H. SCHMIDT, Frankfurt. Ztschr. f. Path. **51**:43, 1938.

Schmidt defines gemmangioma as a tumor originating from capillaries. It differs from hemangioma in that there are no mature vessels present. It contains

an amorphous homogeneous basic material rather than a cellular stroma. The tumor resembles granulation tissue without inflammatory cells. The author describes other forms which contain much of a hyaline or amyloid substance. The production of red blood cells and the ability to phagocytose hemosiderin is taken as proof of the endothelial genesis of these capillary sprouts. The malignant gemmangioma is most frequently found in the extremities. The author concludes that the gemmangioma is the connecting link between hemangioma and angiosarcoma.

OTTO SAPHIR.

PARATHYROID TUMORS AND OSTEITIS FIBROSA CYSTICA. M. MEISEL, Frankfurt. Ztschr. f. Path. **51**:104, 1938.

Meisel illustrates the modern point of view regarding the relation between tumor of the parathyroids and generalized osteitis fibrosa cystica by presenting 3 cases. Most writers recognize changes in the parathyroids as the cause of von Recklinghausen's disease of the bones, while others point out that this disease is caused by a pluriglandular disorder. The author believes that, despite the close interaction between all endocrine glands, the existence of a single endocrine dysfunction is not precluded. According to many opinions, changes in the parathyroids are a significant feature of von Recklinghausen's disease of the bones, and by these the latter is distinguished from other diseases of bone. Many investigators tried to produce von Recklinghausen's disease experimentally by injecting parathyroid extract or by creating hyperacidity of the blood, but they obtained only changes somewhat resembling von Recklinghausen's disease, never the true disease. The cause of von Recklinghausen's disease is apparently not a change in metabolism alone but the presence of associated parathyroid tumor. In the author's first case the blastoma was a lymphangiectatic chief cell adenoma, in the second case a papillary chief cell adenoma and in the third case a mixed adenoma. In all 3 cases only a single tumor of one parathyroid was present.

OTTO SAPHIR.

RESORPTION OF INJECTED TUMOR CELLS OF THE BROWN-PEARCE CARCINOMA OF RABBITS IN IMMUNIZED ANIMALS. B. KARDJIEV, Frankfurt. Ztschr. f. Path. **51**:391, 1938.

To determine whether immunity to tumor in experimental animals is caused by humoral or by histiocytic reactions, Kardjiev observed the results of intravenous injections of the Brown-Pearce carcinoma of rabbits. Rabbits previously immunized by subcutaneous injections of small doses of suspended tumor cells and normal rabbits were given intravenous injections of tumor cells at the same time. These experiments revealed that in the first hours after the intravenous injection the injected tumor cells found their way into the capillaries of the lungs and showed partial or total necrosis. A cellular reaction appeared later, consisting first of eosinophilic leukocytes, which were replaced by granulation tissue, in the immunized animals, while the control animals showed only a very weak reaction. On the second day tumor masses grew in control animals, while the surrounding cellular reaction was completely absent. In the immunized animals the intravenously injected tumor masses became smaller and were finally replaced by histiocytic elements. The cellular reaction was not due to necrosis. It was severe in immunized animals around preserved tumor masses and was completely absent in the control animals around necrotic areas. The author concludes that the destruction of the tumor cells in immunized animals was due to their replacement by mesenchymal elements. This he considers the mechanism of immunity.

OTTO SAPHIR.

Society Transactions

BUFFALO PATHOLOGICAL SOCIETY

ERNEST WITEBSKY, *President*

June 17, 1939

SAMUEL SANES, *Secretary*

HYALINE ADENOMA OF THE CERVIX. NORMAN W. ELTON.

A tumor of the cervix, of a type heretofore not reported, which is termed hyaline adenoma, or Stone's adenoma, and which has been observed in situ for three years, and two other similar lesions lacking the hyaline stroma are presented. The life cycle of the hyaline adenoma began with a proliferation of small cystic cervical glands in a dense hyalinized stroma which comprised 50 per cent of the tissue and in which the disorganized glandular epithelium exhibited some squamous metaplasia. Subsequent biopsies showed disintegration and disappearance of the glands and development of hyperplastic squamous epithelium embedded in an increased hyaline stroma. This lesion has manifested no evidence of malignancy and is believed to be of inflammatory origin, since the patient had had a cervical discharge for ten years. This tumor, as well as the other 2, had originally been diagnosed adenocarcinoma, but the biologic character of each has been that of a benign lesion.

EFFECT OF SULFAPYRIDINE ON THE VIABILITY OF MENINGOCOCCI IN SPINAL FLUID. ERWIN NETER and DAVID H. WEINTRAUB.

Sulfanilamide as well as sulfapyridine (2 - [paraaminobenzenesulfonamide] - pyridine) have proved to be effective chemotherapeutic agents in meningococcic infection, particularly meningococcic meningitis. In an endeavor to analyze the mode of action, experiments were undertaken to test the possible bacteriostatic and bactericidal action on meningococci in spinal fluid. It was found that sulfanilamide in certain concentrations inhibits the growth and causes loss of viability of meningococci in spinal fluid of patients suffering from meningococcic meningitis (Neter, E.: *Proc. Soc. Exper. Biol. & Med.* **38**:37, 1938; **39**:84, 1938). Leukocytes were present in the spinal fluid of these patients. Thus, the question arises whether or not the presence of leukocytes is necessary for the bacteriostatic and bactericidal action of sulfanilamide in this order of experiment. To this end, the following experiments were carried out.

Meningococci grown on ascitic-chocolate agar plates were suspended in normal spinal fluid to a density of approximately 50,000,000 micro-organisms per cubic centimeter. Two strains of meningococci were used, one, freshly isolated from a meningococcic meningitis case, the other one, an old stock culture strain. This suspension was then mixed with equal amounts of (*a*) spinal fluid of patients who were receiving sulfapyridine and (*b*) spinal fluid of patients who were not receiving this drug. The tubes were incubated at 37 C. At various intervals subcultures were made on ascitic-chocolate agar plates, which were incubated at 37 C. for at least seventy-two hours. It was found that when subcultures were made from both tubes within a few minutes meningococci grew out profusely from spinal fluid suspensions containing sulfapyridine as well as from the controls. Subcultures made from three to six hours after meningococci were suspended in spinal fluid containing sulfapyridine in concentrations of 4.1, 5.3, 7 and 16 mg. per hundred cubic centimeters, respectively, either failed to grow out entirely or their

growth was markedly retarded in contradistinction to the controls, which grew out profusely. In two experiments with spinal fluid containing only 1.2 and 3.6 mg. per hundred cubic centimeters of sulfapyridine, meningococci were found to be viable even after being exposed to the action of the drug for eight and five hours, respectively. It is important to state that meningococci lost their viability in control spinal fluid within ten to eighteen hours' incubation at 37 C. This is in sharp contrast to what was observed with spinal fluid from patients with meningococcic meningitis prior to specific treatment: In such spinal fluid meningococci not only remained viable for at least eighteen to twenty-four hours but often showed a marked increase.

These experiments show that meningococci may lose their viability in spinal fluid when exposed to the action of sulfapyridine. Since such spinal fluid was practically free from leukocytes, it may be concluded that leukocytes are not absolutely necessary, although, when present, they may contribute largely to the effect of sulfanilamide or sulfapyridine. It is important to emphasize that meningococci in spinal fluid free from leukocytes and globulin may lose their viability quite rapidly. It is possible that the factors which lead to the death of meningococci in normal spinal fluid may contribute to or, according to Mellon, even potentiate the bactericidal action of sulfapyridine. In considering the mode of action of sulfanilamide and related compounds it is important to keep in mind that the action of these chemotherapeutic substances depends on three factors: (1) the concentration and the chemical composition of the chemotherapeutic substance, (2) the species or type and the number of micro-organisms and (3) the environmental influences. When these factors inhibit growth or promote death, they may add to or potentiate the action of the chemotherapeutic substance, while when growth-promoting they may counteract the action of the drug.

EFFECT OF SPECIFIC TREATMENT ON THE SERUM OF PATIENTS SENSITIVE TO RAGWEED. CARL E. ARBESMAN.

Although the specific therapy of hay fever due to pollen has been successfully and universally used since 1911, its exact mechanism has as yet not been clearly elucidated. Some observers believe that true desensitization takes place, while others believe that the treatment may immunize the patient. Recently Cooke and co-workers demonstrated that an inhibiting antibody is produced as one result of specific therapy, which acts to prevent the reaction between reagin and allergin. In view of the discrepancies apparent in previously reported studies, the effect of specific treatment on the reagin content of the serum of naturally sensitive persons were reinvestigated. An attempt was also made to repeat the recent experiments of Cooke.

Specimens of the serum of 59 patients taken both before and after treatment were available for study. These specimens were all stored at —20 C.

The reagin content of the serum before and after treatment was determined by sensitizing comparable sites in at least 2 nonatopic persons, with serial twofold dilutions. Care was taken to carry out this titration of the 2 serum specimens simultaneously, in the same recipient. Twenty-four to forty-eight hours after passive sensitization, all sites were tested with a 1:100 dilution of a standard pollen extract. The highest dilution of serum which just sufficed to sensitize the skin was taken as the titer of the serum. Essentially the same technic as described by Cooke and co-workers was used in an attempt to demonstrate the inhibitor substance.

The therapeutic results were apparently not significantly altered by age, sex, duration of symptoms prior to treatment, the amount of treatment or its duration. Similarly, the quantitative change in the reagin content of the serum appeared to be independent of these factors.

Of the 59 patients studied, 35 showed no appreciable change in reagin content; 14 showed a slight increase of reagin after treatment and 10 a decrease. Among 3 of these 10, the most marked decrease was noted in those who had received rela-

tively large amounts of treatment. Although the number of patients is too small for one to draw any definite conclusions, the results suggested that when treatment is given repeatedly over a sufficiently long period of time there is a tendency toward reduction of the amount of reagin in the serum. However, this decrease in reagin content is not essential for good clinical results. Many of the patients whose serum showed either only a slight increase or no change at all in the reagin content had just as good clinical results.

In an attempt to verify the findings of Cooke and co-workers on the inhibitor substance, the results were irregular and inconclusive, even in the serum of patients who had been heavily treated over a period of two years.

No significant change in the complement-fixing or precipitating activity could be demonstrated in the serum even of heavily treated patients.

DIFFUSE MUCOID CARCINOMATOSIS OF THE LUNGS. K. L. TERPLAN and EMERSON HOLLEY.

WASHINGTON SOCIETY OF PATHOLOGISTS

H. E. RAGLE, *President*

Regular Meeting, Oct. 7, 1939

V. H. CORNELL, *Secretary*

FILARIASIS OF THE TESTICLE AND EPIDIDYMIS. H. E. RAGLE.

Sections of a testicle and epididymis were presented from a white man aged 36, a laborer, who had had recurring attacks of pain in the right testicle and occasional vomiting spells for the past three months. There was no history or evidence of venereal disease. This material was forwarded from Samoa. Physical examination showed the right testicle moderately enlarged and the epididymis enlarged and nodular. Roentgen examination of the chest gave negative results. The prostate was normal. The right testicle with the epididymis measured 6.5 by 4 by 3 cm. On section the tunica albuginea was markedly thickened, and the testicle proper was atrophic, measuring only 2 cm. in diameter. The epididymis, which occupied the upper half of the specimen, was hard and nodular. Microscopic examination revealed a markedly thickened and vascular tunica albuginea and cross sections of gravid female Wuchereria bancrofti in dilated lymph vessels. The testicle was atrophic, and in a few areas there were dilated lymph vessels in the connective tissue stroma, which contained filaria. The epididymis also showed filaria. The diagnosis was filariasis with atrophy of the testicle.

FETAL ADENOMA OF THE THYROID. OSCAR B. HUNTER.

Sections were presented together with a gross specimen from a white woman 44 years of age who was admitted to the hospital on July 20, 1939, complaining of difficulty in swallowing and nervousness. The family history was noncontributory. Her illness apparently had its inception about ten years prior to examination, when an enlargement of the neck occurred, causing discomfort in swallowing. In 1935 the condition became worse, and at times dyspnea took place. After April 1939 the symptoms of pressure increased, causing great difficulty in swallowing and hoarseness. There was a history of palpitation, occurring infrequently. Her last menstrual period was in May 1939. The patient was obese. The blood pressure was 125 systolic and 85 diastolic; the temperature, 98.6 F.; the pulse rate 98, and the respiratory rate, 26. The only positive finding was bilateral enlargement of the thyroid gland. Subtotal thyroidectomy was performed on July 21, 1939. A note at this time stated that the right lobe extended around

in back of the trachea, holding it in a fixed position. An uneventful recovery followed, and the patient was discharged on July 25. Histologically this tumor was classed as an adenoma, probably of fetal type.

UNDIFFERENTIATED SMALL CELL CARCINOMA OF THE SMALL INTESTINE. V. H. CORNELL.

Tissues were presented from a 43 year old white man, a mattress maker. The past and family history were not given. The onset of symptoms began about the middle of July 1938, with weakness, frequency of urination, shortness of breath, night sweats and no pain but drowsiness in the daytime. He was treated with prostatic massage, and a cystoscopic examination was made in September or October 1939. Pyelograms made on Feb. 15, 1939, were normal. Exploratory laparotomy was done February 17, at which time an inoperable mass was found in the midline and to the right in the abdomen, which involved the small intestine, cecum and omentum. A biopsy was reported as showing an inflammatory reaction with no malignant development apparent in the specimen. The blood on March 22 showed: sugar, 72 mg. and nonprotein nitrogen, 32 mg. per hundred cubic centimeters; red blood cells, 2,416,000 per cubic millimeter; hemoglobin, 60 per cent; white cells, 8,500 per cubic millimeter, with 87 per cent neutrophils, 3 per cent monocytes and 10 per cent lymphocytes. The urine was normal. Autopsy, April 19, one hour and five minutes post mortem, disclosed a fibrous mass of coiled intestine involving the lower end of the jejunum, measuring approximately 10 inches (25 cm.) in length and 3 inches (7.5 cm.) in diameter. On gross section the intestinal lumen was patent throughout the mass, and there was no dilatation immediately above the mass; the portion of the intestine involved was fibrous and firm, with thick walls, which were friable and easily penetrated in places, but neither the serosa nor the mucosa appeared involved in the abnormal thickening of the muscle layers. A few neighboring mesenteric nodes were small, firm, discrete and on section firm and grayish white. Except for fibrous adhesions to the omentum and adjacent coils of intestine there were no evidences of extension or metastases to other organs. The transition from the mass to the normal intestine above and below was abrupt, with no apparent acute inflammatory process. The rest of the gastrointestinal tract and all other thoracic and abdominal organs showed nothing pertinent. The head was not opened. The prosector considered the possibility of this condition being (1) an argentaffine tumor or (2) sclerosing ileitis.

Histologically, the regional lymph nodes showed invasion by the tumor, but all other organs were not so involved. The tumor itself was generally limited to the muscle coat with some necrosis and inflammation of the mucosa and submucosa. The tumor cells varied from small round to large multinuclear polyhedral ones. There was no definite architecture, though in places a stalklike structure was noted, probably due to necrosis, with some persistent cells near the vascular supply. By Fontana stain no granules were seen within the cells. Reticulum stains showed islands of cells with the reticulum not in intimate association with them. For want of a better designation, this has been classed as an undifferentiated small cell carcinoma of the small intestine.

GRANULOSA CELL TUMORS OF THE OVARY. GEORGE TOLSTOI.

Sections were presented of an ovary removed from a 28 year old white woman, who was admitted to the hospital April 27, 1939, for removal of an ovarian cyst. Her menstrual history began at the age of 14; the periods were regular, with a twenty-eight day cycle, but menstruation was prolonged for six days. She had been married for two and one-half years, and although she had desired children she had not become pregnant. In April excessive menorrhagia was noted. This persisted for six days and subsided. Soreness was noted April 20, with pain in both lower quadrants of the abdomen. A mass was found in the lower left

quadrant, about the size of a grapefruit. The left ovary was removed April 27; convalescence was uneventful, and the menstrual periods have been regular again. The histologic picture together with the clinical findings favor a diagnosis of granulosa cell tumor.

(In discussion the spindle shape of many of the cells was remarked on, the possibility of an arrhenoblastoma considered and the relation of these tumors to the menstrual history discussed. The general opinion favored a diagnosis of granulosa cell tumor.)

A Case of Chondromyxosarcoma. J. H. McNinch.

In 1938 a white man aged 62 years was admitted to the hospital for excision of several subcutaneous nodules; one was on the perineum; the others were on the face. The patient was born in 1876 and began to complain of pain in the upper third of the left fibula in 1898. In 1922 he was operated on and told that a portion of the fibula was removed and that the diagnosis was sarcoma. There was recurrence locally in soft tissues, treated by roentgen rays and excision on several occasions and resulting finally in amputation of the leg above the knee in 1931. The first tissue, from the leg, was received by the Army Medical Museum in 1929, at which time a diagnosis of chondromyxosarcoma was made. A possible metastatic lesion in the subcutaneous tissues of the neck was reported in 1929, but no examination of tissue was made at that time. In 1938 this lesion was excised, together with other nodules in the subcutaneous tissues of the face, neck and perineum, and all were found to be metastatic lesions. In July 1939 roentgen examination showed metastasis to the right clavicle. This was apparently the first metastatic lesion in bone. The patient is now alive, seventeen years after the primary removal of the tumor, in 1922, and ten years after the appearance of the first metastatic lesion, in 1929.

Book Reviews

Handbuch der Virusforschung. Edited by Prof. Dr. R. Doerr, Basel, and Prof. Dr. C. Hallauer, Berne, Switzerland. Volume 1. Paper. Price 66 reichsmarks. Pp. 546, with 71 illustrations, some in color. Vienna: Julius Springer, 1938.

This is the first of two volumes of a handbook prepared by several Swiss, German, British and American collaborators. Some of the chapters are written in German, some in English. The purpose of the book is to cover the broad aspects of the study of viruses beyond the circumscribed field of the practical application to virus diseases. The interrelations with physics, with the chemistry of proteins, with the general physiology of cells and with genetics, the methods of study and the problems involved were to be considered to a greater extent than had been done heretofore. A study of the volume proves that these objectives have been accomplished to a remarkable degree. The consideration of special details of each subject does not obscure the wider aspects and relations. The facts concerning viruses are treated as a part of the natural sciences.

The method of synthesis is at its best in the first part, on the history and the problems of the study of viruses, by R. Doerr. The subject is treated as the history of problems as they have evolved since 1892, when Iwanowsky reported his filtration experiments. The presentation is profound, philosophic, stimulating and highly critical. The 109 pages should be read by every one interested in natural sciences and obviously by every microbiologist.

Elford presents in the first chapter of the second part the methods for determining the sizes of viruses and of bacteriophages, on 94 pages. This English chapter deals with methods, their technics, their application and the results. Ultrafiltration and centrifugation, analysis and ultraviolet photography are treated clearly and fully. A short chapter by Haitinger presents the technic and the results of research by means of fluorescence microscopy. Another by Kaiser deals with the staining of viruses and has some excellent colored illustrations. Findlay is the author of a chapter on inclusion bodies and their relationship to viruses. Cytoplasmic and intranuclear inclusions and their significance are discussed. Hallauer is responsible for a chapter on the cultivation of viruses in tissue explants and Burnet of Melbourne for one on the growth of viruses on the chorioallantois of the chick embryo, a method based on the discovery of Woodruff and Goodpasture. The last chapter, by Stanley of the Rockefeller Institute for Medical Research, on the biochemistry and biophysics of viruses, deals with the inactivation of viruses by different agents, with the concentration and purification of these agents and with their chemical and physical properties. Each chapter is followed by a comprehensive bibliography. As evidenced by the names of the authors, every subject was dealt with by a master. The paper, the print and the illustrations are excellent.

There is little doubt that the book will be invaluable to all who are interested in viruses.

The second volume will deal with viruses as agents of infection, with natural susceptibility to viruses, with acquired immunity and with experimental studies on viruses that are pathogenic for plants. The index for the whole work will be in the second volume.

Clinique et pathologie comparée. Vénéréologie—cancérologie—dermatoses, médecine générale—phyto-pathologie. L. Bory. Price 50 francs. Pp. 239. Paris: Masson & Cie, 1939.

Bory, a dermatologist, has been interested for many years in veterinary medicine. He has studied diseases of the skin in animals, utilizing the material of the veterinary school in Paris. In 1936 he published a book on comparative

dermatology in collaboration with Henry of the veterinary school. He was prompted to write the present book by the realization that while there are treatises on human pathology, on veterinary medicine and on plant pathology, none is available that deals synthetically with the pathology of man, animals and plants. The basic thesis is that the diseases of man, animals and to some extent also plants are similar, that they are controlled by the same fundamental laws and that by their study the different branches of pathology will be mutually enriched. Among students of human pathology there has been a lack of interest in veterinary medicine. To stimulate that interest is the main purpose of the book, and it can be stated at the outset that the aim has been achieved. The subjects discussed in the book are: spontaneous and experimental venereal diseases of animals; the so-called syphiloid of the cat (better known as cancroid of the lip of the cat) and its similarity to Hodgkin's lymphogranuloma in man; a comparison of benign and malignant tumors and tumors thought to be due to parasites, in man and animals; scabies and parasitic infections of the skin; the tuberculous infections, including the so-called paratuberculous infections of the skin; leprosy; typical cutaneous lesions as, for instance, eczema, neurodermatitis and lichen; the comparative pathology of infectious jaundice and that of epilepsy. As is evident from this list, the topics are rather disconnected. The conditions are compared in man and animals and the relations brought out, though not always convincingly. One cannot fail to notice that the enthusiasm of the author to find similarities tends to lead him astray. Some of his correlations do not impress one as having a sound foundation. That is frequently the case with pioneers, and as such, it does not detract too much from the importance of the book. That there are diseases common to man and animals is well brought out in the ninth chapter, in which the professional afflictions of veterinarians are presented. An introduction to the pathology of plants concludes the book.

It is sometimes misleading when in describing diseases of animals terms are used whose meaning in human pathology is entirely different. In future comparative studies that error will have to be corrected. Some defects are due to an improper use of terms—e. g., "active immunity" and "passive immunity," which have a well established meaning and which the author uses, unfortunately, in an entirely different sense. Another defect is a scanty and obviously defective bibliography. An index is missing.

Symposium on the Synapse. Herbert S. Gasser, Joseph Erlanger, Detlev W. Bronk, Rafael Lorente de Nó and Alexander Forbes. (Reprinted from the *Journal of Neurophysiology* [**2**:361, 1939].) Cloth. Price, $2. Pp. 113. Springfield, Ill., and Baltimore, Md.: Charles C. Thomas, Publisher, 1939.

This book contains the contributions, revised, to the symposium on synaptic transmission held during the 1939 meeting of the American Physiological Society. The speakers and the subjects covered were as follows: Herbert S. Gasser, "Axons as Samples of Nervous Tissue"; Joseph Erlanger, "The Initiation of Impulses in Axons"; Detlev W. Bronk, "Synaptic Mechanisms in Sympathetic Ganglia"; Rafael Lorente de Nó, "Transmission of Impulses Through Cranial Motor Nuclei," and Alexander Forbes, "Problems of Synaptic Function."

From the list of subjects it will be obvious that the discussion was not narrowly confined to the mechanism by which nerve impulses are transmitted from one neuron to the next across a point of contact called the synapse, but that instead the data concerning the physiology of such diversified structures as axons, sympathetic ganglions and cranial motor nuclei were organized as an indirect attack on the mechanism of synaptic transmission, which has eluded direct experimentation. Doubt was thrown on the common belief that the manifestations of synaptic activity are exclusively found at the junction between neurons by the presentation of examples of synaptic peculiarities which may appear in polarized nerve fibers, by the recognition of increased or decreased excitability of axons varying with

the electrical sign of the after-potential, which suggests a mechanism for inhibition, and by the emphasis on internuncial activity involving closed "self re-exciting chains." It was shown that many central phenomena may be explained in terms common to the peripheral nervous system.

Finally, the overheated controversy between the chemical theory, according to which an exciting substance, perhaps acetylcholine, is released at the terminations of a neuron on the dendrites or cell body of a successive neuron, and the electrical theory, according to which the physical effects of a potential gradient will excite a synapse, was largely avoided. Gasser, Erlanger and Lorente de Nó considered electrical phenomena exclusively, Forbes stressed the possibility of chemical transmission, and Bronk utilized both, in the attempt to portray the function of the sympathetic ganglions. Each of them acknowledged the existence of both chemical and physical factors. Indeed, it was suggested by Forbes that electrochemical events exist in such a relation that the electrical or chemical properties of transmission emerge according to the type of experiment performed, much as experiments on light in physical laboratories show corpuscular or wave action according to the type of experiment performed.

La recherche de la paternité par les groupes sanguins. Étude technique et juridique. L. Christiaens. Paper. Price 26 francs. Pp. 108. Paris: Masson & Cie.

Christiaens is a member of the Institute of Legal and of Social Medicine at Lille, France, where he and a group of investigators have devoted themselves to the study of blood groups with particular attention to the use of blood grouping for exclusion of paternity. The chief of the institute, J. Leclercq, wrote an introduction to the monograph. It seems that certain special features of the French legal code with regard to the problem of paternity make the medicolegal application of blood groups difficult. The French students of blood groups find it hard to arouse the interest of jurists. The purpose of this monograph is to create interest in, and to enhance appreciation of, the possibility of excluding paternity by blood grouping. First comes a discussion of the general aspects of blood groups; the problems of inheritance are clearly presented. The technical details and the legal aspects of blood grouping are discussed, with special attention to conditions in France. Christiaens studied the legal status of blood grouping in most countries, and the results are instructively summarized. In the concluding chapter the outlook for the future of the procedure in France and its social implications are taken up. The importance of expert technic and of sufficient experience in interpreting the results is stressed. The handy little volume can be recommended to all interested in blood grouping and its medicolegal applications.

Epidemic Encephalitis. Etiology, Epidemiology, Treatment. Third Report by the Matheson Commission, Willard C. Rappleye, chairman. Cloth. Price $3. Pp. 493. New York: Columbia University, 1939.

This is the third summary of published data relating to the epidemiologic aspects, causes and treatment of epidemic encephalitis. The first report was published in 1929, the second in 1932. The present report is a useful reference book for workers on any of the various forms of epidemic encephalitis or allied diseases. It covers as fully as possible the work done anywhere on the three phases of encephalitis mentioned in the title during the period from the beginning of 1930 through the first half of 1937. Many important publications of the second half of 1937 and the first half of 1938 are included also. There are author and subject indexes.

Books Received

ELECTROGRAPHIC PATTERNS. Arlie R. Barnes, M.D., Mayo Clinic, Rochester, Minn. Cloth. Pp. 197, with 94 illustrations. Price $5. Springfield, Ill., and Baltimore, Md.: Charles C. Thomas, Publisher, 1939.

THE HOSPITAL CARE OF NEUROSURGICAL PATIENTS. Wallace B. Hamby, M.D., F.A.C.S., Associate Professor of Neurology and Instructor in Surgery, University of Buffalo School of Medicine, Buffalo. Cloth. Pp. 118, with 24 illustrations. Springfield, Ill., and Baltimore, Md.: Charles C. Thomas, Publisher, 1939.

THE DIAGNOSIS AND TREATMENT OF DISEASES OF THE ESOPHAGUS. Porter P. Vinson, B.S., M.A., M.D., D.Sc., F.A.C.P., Professor of Bronchoscopy, Medical College of Virginia, Richmond, Va. Cloth. Pp. 224, with 98 illustrations. Price $4. Springfield, Ill., and Baltimore, Md.: Charles C. Thomas, Publisher, 1939.

HANDBOOK OF PUBLIC HEALTH BACTERIOLOGY AND CHEMISTRY. GENERAL INFORMATION REGARDING EPIDEMIOLOGY, COLLECTION AND SHIPMENT OF SPECIMENS, AND BACTERIOLOGIC, SEROLOGIC AND CHEMICAL PROCEDURES, 1939. Edited by M. S. Marshall. Paper. Pp. 150. San Francisco: J. W. Stacey, Inc., 1939.

PATHOLOGY. AN INTRODUCTION TO MEDICINE AND SURGERY. J. Henry Dible, M.B., F.R.C.P., Professor of Pathology, University of London; and Thomas B. Davie, M.D., M.R.C.P., Professor of Pathology, University of Liverpool. Cloth. Pp. 931, with 374 illustrations. Price $10. Philadelphia: P. Blakiston's Son & Co., 1939.

HARVEY CUSHING'S SEVENTIETH BIRTHDAY PARTY. APRIL 8, 1939. SPEECHES, LETTERS, TRIBUTES. Published for the Harvey Cushing Society. Cloth. Pp. 146, with frontispiece. Price $3. Springfield, Ill.: Charles C. Thomas, Publisher, 1939.

INDEX TO VOLUME 28

The asterisk (*) preceding the page number indicates an original article in the Archives. Subject entries are made for all articles. Author entries are made for original articles and society transactions. Book Reviews, Obituaries and Society Transactions are indexed under these headings in their alphabetical order under the letters B, O and S, respectively.